

TRADE SECRET

Unpublished Work
Copyright ©2011 E.I. du Pont de Nemours and Company

STUDY TITLE: H-28548: Absorption, Distribution, Metabolism, and Elimination in the Mouse

TEST GUIDELINES: U.S. EPA Health Effects Test Guidelines
OPPTS 870.7485 (1998)

AUTHOR: William J. Fasano, Sr., B.S.

ORIGINAL REPORT

COMPLETED: November 3, 2010

REPORT REVISION 1

COMPLETED: April 21, 2011

PERFORMING LABORATORY: E.I. du Pont de Nemours and Company
DuPont Haskell Global Centers for
Health & Environmental Sciences
P.O. Box 50
Newark, Delaware 19714
U.S.A.

E.I. du Pont de Nemours and Company
DuPont Experimental Station (CCAS)
Wilmington, Delaware 19803
U.S.A.

LABORATORY PROJECT ID: DuPont-18647-1017

WORK REQUEST NUMBER: 18647

SERVICE CODE NUMBER: 1017

SPONSOR: E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

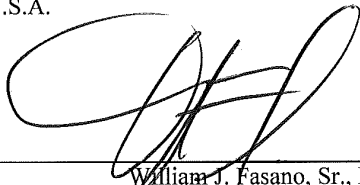
GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with U.S. EPA TSCA (40 CFR part 792) Good Laboratory Practice Standards, which are compatible with current OECD Good Laboratory Practices, except for the item documented below. The item listed does not impact the validity of the study.

1. Qualitative analysis of urine samples for structure confirmation and elucidation was conducted on a non-GLP Liquid Chromatography/Mass Spectrometry (LC/MS) system. However, the identity of the parent analyte, the only analyte detected, was confirmed in urine samples using the test substance H-28548, which had a matching nominal mass-to-charge (m/z) ratio of approximately 329.

Sponsor: E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

Study Director: _____


William J. Fasano, Sr., B.S.
Senior Research Toxicologist

21-APR-2011
Date

Sponsor: _____

Sponsor Representative

Date


QUALITY ASSURANCE STATEMENT

Work Request Number: 18647
Service Code Number: 1017

Key inspections for the above referenced study were completed by the Quality Assurance Unit of DuPont Haskell and the findings were submitted on the following dates:

| <i>Audit Dates</i> | <i>Date Reported to Study Director</i> | <i>Date Reported to Management</i> |
|---|---|---|
| <u>Protocol:</u> March 17, 2010 | March, 17, 2010 | March, 17, 2010 |
| <u>Conduct:</u> March 31, 2010 June 09, 2010 | March 31, 2010 June 09, 2010 | March 31, 2010 June 09, 2010 |
| <u>Report/Records:</u> October 08, 11-13, 2010 | October 13, 2010 | October 14, 2010 |
| <u>Sponsor Edits 1:</u> October 28, 2010 | October 28, 2010 | October 28, 2010 |
| <u>Report Revision 1:</u> April 11, 2011 | April 11, 2011 | April 11, 2011 |


Reported by: _____

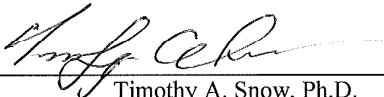

Antonio Pedulla
Quality Assurance Auditor



Date

CERTIFICATION

We, the undersigned, declare that this report provides an accurate evaluation of data obtained from this study.

LC/MS/MS  For MPM 20-APR-2011
Quantitation by: Michael P. Mawn, Ph.D.
Senior Research Chemist Date

LC/MS Metabolite ID by:  21-APR-2011
Timothy A. Snow, Ph.D.
Senior Research Chemist Date

Reviewed and Approved by:  19-APR-2011
Gary W. Jepson, Ph.D.
Manager Date

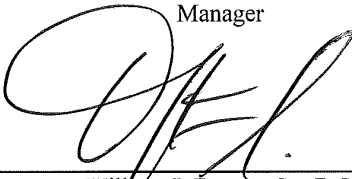
Issued by Study Director:  21-APR-2011
William J. Fasano, Sr., B.S.
Senior Research Toxicologist Date

TABLE OF CONTENTS

| | Page |
|---|-----------|
| GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT | 2 |
| QUALITY ASSURANCE STATEMENT | 3 |
| CERTIFICATION..... | 4 |
| TABLE OF CONTENTS | 5 |
| LIST OF TABLES | 6 |
| LIST OF FIGURES | 6 |
| LIST OF APPENDICES | 7 |
| STUDY INFORMATION | 8 |
| REPORT REVISION 1 | 9 |
| SUMMARY | 9 |
| INTRODUCTION..... | 10 |
| OBJECTIVE | 10 |
| ANIMAL WELFARE ACT COMPLIANCE | 10 |
| MATERIALS AND METHODS | 10 |
| A. Test Guidelines | 10 |
| B. Test Substance | 11 |
| C. Test System..... | 11 |
| D. Animal Husbandry | 11 |
| 1. Housing | 11 |
| 2. Environmental Conditions..... | 11 |
| 3. Feed and Water | 11 |
| 4. Animal Health and Environmental Monitoring Program | 11 |
| E. Pretest Period | 12 |
| F. Assignment to Groups..... | 12 |
| G. Dose Preparation, Analysis, and Rates | 12 |
| H. In-Life Phase | 13 |
| 1. Material Balance and Tissue Distribution | 13 |
| I. Quantitation of H-28548 | 14 |
| 1. Sample Receipt..... | 14 |
| 2. Sample Preparation Procedure (dose solution and urine samples) | 14 |
| 3. Sample Preparation Procedure (cage wash samples) | 14 |
| 4. Sample Preparation Procedure (feces samples)..... | 14 |
| 5. Stock Solutions and Calibration Standards | 14 |
| 6. Instrument and Conditions | 15 |
| 7. Quantitation..... | 16 |
| J. Identification of Metabolites..... | 16 |
| 1. Liquid Chromatography/Mass Spectrometry (LC/MS)..... | 16 |
| 2. Data processing | 17 |

| | |
|--|-----------|
| STATISTICAL AND DATA ANALYSIS | 17 |
| RESULTS AND DISCUSSION | 18 |
| A. Quantitation of H-28548 by LC/MS/MS | 18 |
| 1. Calibration Standard Curve | 18 |
| 2. Limit of Detection and Limit of Quantitation | 18 |
| 3. Chromatographic Results (urine, cage wash, and dose samples) | 19 |
| 4. Chromatographic Results (feces samples) | 19 |
| 5. Fortification QC Sample Results | 19 |
| B. Dose Formulation Concentration, Animal Body Weights, Dosing Information | 19 |
| C. Urine Data | 20 |
| D. Feces Data | 20 |
| E. Material Balance | 20 |
| F. Metabolite Identification | 21 |
| G. Elimination Half-Life ($T_{1/2}$) | 21 |
| CONCLUSIONS | 21 |
| RECORDS AND SAMPLE STORAGE | 22 |
| REFERENCES..... | 22 |
| TABLES..... | 23 |
| FIGURES..... | 27 |
| APPENDICES | 38 |

LIST OF TABLES

| | Page |
|---------|--|
| Table 1 | Mouse urine sample fortification QC results for H-28548.....25 |
| Table 2 | Mouse feces sample fortification QC result for H-28548.....25 |
| Table 3 | Dosing information.....25 |
| Table 4 | Urine, cumulative percent of dose.....26 |
| Table 5 | Feces, cumulative percent of dose.....26 |
| Table 6 | Material balance, percent of dose.....26 |

LIST OF FIGURES

| | Page |
|----------|--|
| Figure 1 | Calibration curve for H-28548.....29 |
| Figure 2 | The LC/MS/MS chromatograms for a) lowest calibration standard at 2.5 ng/mL, b) urine control matrix sample, c) low level 400 ng/g fortification QC sample with preparation |

| | | |
|----------|--|----|
| | factor 40x, and d) a 24-hour urine study sample from animal 001M, which had a total dilution factor of 1480x and final concentration of 14,800 ng/g..... | 30 |
| Figure 3 | The LC/MS/MS chromatograms for a) lowest calibration standard at 2.5 ng/mL, b) feces control matrix sample, c) low level 250 ng/g fortification QC sample that had a preparation factor of 6.14x, and d) a 12-hour feces study sample from animal 001M, which had a 34x dilution factor and final concentration of 775 ng/g..... | 31 |
| Figure 4 | Urine, cumulative percent..... | 32 |
| Figure 5 | Feces, cumulative percent..... | 33 |
| Figure 6 | Material Balance, percent of dose..... | 34 |
| Figure 7 | Reconstructed m/z 329 + 659 ion chromatograms characteristic of H-28548-dosed female mouse urine (6 hours after administration) – top and control mouse urine fortified with H-28458 test substance -bottom..... | 35 |
| Figure 8 | ESI negative mass spectra of H-28548 observed in dosed female mouse urine (6 hours after administration)–top; and control urine fortified with H-28548 test substance – bottom..... | 36 |
| Figure 9 | ESI negative daughter ion mass spectra of H28548 observed in dosed female mouse urine (6 hours after administration)–top; and control mouse urine fortified with H-28548 test substance – bottom..... | 37 |

LIST OF APPENDICES

| | | |
|------------|-------------------------------|------|
| | | Page |
| Appendix A | Certificate of Analysis | 40 |
| Appendix B | Dosing Information..... | 42 |
| Appendix C | Urine Data..... | 44 |
| Appendix D | Feces Data..... | 49 |
| Appendix E | Cage Wash Data..... | 54 |
| Appendix F | Material Balance | 56 |
| Appendix G | Elimination Half-Life..... | 58 |

STUDY INFORMATION

Substance Tested:

- HFPO Dimer Acid Ammonium Salt
- 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propionic acid, ammonium salt
- 62037-80-3 (CAS Number)
- H-28548

Haskell Number: 28548

Composition: Proprietary

Purity: 84%

Physical Characteristics: Clear and colorless liquid

Stability: The test substance appeared to be stable under the conditions of the study; no evidence of instability was observed.

Study Initiated/Completed: March 16, 2010 / (see report cover page)

Experimental Start/Termination: March 16, 2010 / June 11, 2010

In-Life Initiated/Completed: March 31, 2010 / April 7, 2010

Notebook Number(s): E-114321-AM, E-114321-AI, E-98524-GE

REPORT REVISION 1

The elimination half-life ($T_{1/2}$) for H-28548 in male and female mice, following a single oral dose at 3 mg/kg, was estimated and reported.

SUMMARY

The absorption, distribution, metabolism, and elimination of H-28548 were investigated in the Crl:CD1(ICR) mouse. H-28548 was administered in water to 5 male and 5 female mice as a single oral dose at a target dose level of 3 mg H-28548/kg bodyweight (bw) and a dose volume of 10 mL/kg bw. Mice were housed individually in metabolism units and urine and feces were collected on dry ice predose and postdose at 0-6 hours, 6-12 hours, 12-24 hours, and every 24 hours until 168 hours post-dose. At 168 hours post-dose, mice were asphyxiated by exposure to carbon dioxide and then sacrificed by exsanguination. H-28548 was quantitated in urine, feces, and cagewash by liquid chromatography tandem mass spectrometry (LC/MS/MS). Urine samples were further evaluated by LC/MS to confirm the identity of the parent analyte and determine if H-28548 was eliminated metabolized or unmetabolized.

Following oral administration of H-28548 in water, $30.8\% \pm 5.37\%$ and $39.3\% \pm 5.58\%$ of the administered dose was accounted for in urine (0-12 hours) from male and female mice, respectively. At the conclusion of the study (168 hours post-dose), the total accumulated amount of H-28548 detected in urine was $89.5\% \pm 6.91\%$ and $91.5\% \pm 6.04\%$ of the administered dose for male and female mice, respectively.

Elimination of H-28548 via urine accounted for a majority of the administered dose for both male and female mice; minor levels of H-28548 detected in feces from male ($2.00\% \pm 1.01\%$) and female mice ($1.91\% \pm 0.85\%$) were likely contamination from urine.

Cagewash, which is composed of dried excreta (urine and feces), accounted for $9.64\% \pm 3.99\%$ and $6.25\% \pm 3.16\%$ of the administered dose for male and female mice, respectively.

Following oral dosing with H-28548 in water and a 168 hour post-dose collection period, $101.2\% \pm 3.22\%$ and $99.7\% \pm 2.95\%$ of the administered dose was recovered from male and female mice, respectively.

Samples of urine evaluated using LC/MS were found to contain only the parent substance, H-28548. This finding, taken with recovery of the administered dose in urine, confirms that H-28548 was rapidly absorbed and eliminated unmetabolized following oral dosing in the mouse.

The elimination half-life ($T_{1/2}$) for H-28548 in male and female mice, following a single oral dose at 3 mg/kg, was estimated to be 21 and 18 hours, respectively.

INTRODUCTION

The data from this study provides basic information on the absorption, distribution, metabolism, and elimination (ADME) of H-28548 following oral dosing in the mouse.

OBJECTIVE

The objective of this study was to determine the ADME of H-28548 in the mouse following a single oral dose of H-28548 in water. Use of a non-radiolabeled test substance for determining a material balance and metabolite identification in the mouse is justified based on results from an *in vitro* metabolism experiment with rat hepatocytes and rat oral and rat and monkey intravenous dose kinetic studies, which suggests that H-28548 is not metabolized and is eliminated rapidly.^(1,2,3,4)

ANIMAL WELFARE ACT COMPLIANCE

This study complied with all applicable sections of the Final Rules of the Animal Welfare Act regulations (9 CFR) and the Guidelines from the Guide for the Care and Use of Laboratory Animals (NRC 1996). All studies conducted by or for DuPont Haskell adhere to the following principles:

- The sponsor and/or the study director ensures that the study described in this report does not unnecessarily duplicate previous experiments, and is in compliance with the DuPont Policy on Animal Testing.
- Whenever possible, procedures used in this study have been designed to implement a reduction, replacement, and/or refinement in the use of animals in an effort to avoid or minimize discomfort, distress or pain to animals.
- DuPont Haskell policy is that animals experiencing severe pain or distress that cannot be relieved are painlessly euthanized, as deemed appropriate by the veterinary staff and study director or appropriate designee.
- Methods of euthanasia used during this study were in conformance with the above referenced regulation and the recommendations of the American Veterinary Medical Association (AVMA), 2007 Guidelines on Euthanasia.
- DuPont Haskell is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) International.

MATERIALS AND METHODS

A. Test Guidelines

The study design complied with the following test guideline:

- U.S. EPA, OPPTS 870.7485. Metabolism and Pharmacokinetics, Health Effects Test Guidelines (1998)

B. Test Substance

The test substance (CAS registry number 62037-80-3) was supplied by the sponsor and assigned Haskell number 28548.

C. Test System

Male and female Crl:CD1(ICR) mice were obtained from Charles River Laboratories, Inc. (Raleigh, North Carolina, U.S.A.).

The Crl:CD1(ICR) mouse was chosen for this study because of the extensive experience with this strain and its suitability with respect to longevity, sensitivity, and low incidence of spontaneous diseases. Furthermore, the Crl:CD1(ICR) mouse has been used previously for toxicokinetic and toxicity testing of this chemical.

Each animal was assigned a unique identification number to be used throughout the study. The last 3 digits of the animal identification number were marked on the tail of each animal in indelible ink.

D. Animal Husbandry

1. Housing

During the pretest period, animals were housed individually in solid bottom caging with bedding. Animals were moved to metabolism units for the in-life phase of the study.

2. Environmental Conditions

Animal rooms were maintained at a temperature of 18-26°C (64-79°F) and a relative humidity of 30-70%. Animal rooms were artificially illuminated (fluorescent light) on an approximate 12 hour light/dark cycle.

3. Feed and Water

All animals were provided tap water *ad libitum* and fed PMI[®] Nutrition International, LLC Certified Rodent LabDiet[®] 5002 *ad libitum*. When housed in metabolism units, feed was supplied as ground chow.

4. Animal Health and Environmental Monitoring Program

As specified in the DuPont Haskell animal health and environmental monitoring program, the following procedures are performed periodically to ensure that contaminant levels are below those that would be expected to impact the scientific integrity of the study:

- Water samples are analyzed for total bacterial counts, and the presence of coliforms, lead, and other contaminants.

- Samples from freshly washed cages and cage racks are analyzed to ensure adequate sanitation by the cagewashers.

Certified animal feed is used, guaranteed by the manufacturer to meet specified nutritional requirements and not to exceed stated maximum concentrations of key contaminants, including specified heavy metals, aflatoxin, chlorinated hydrocarbons, and organophosphates. The presence of these contaminants below the maximum concentration stated by the manufacturer would not be expected to impact the integrity of the study.

The animal health and environmental monitoring program is administered by the attending laboratory animal veterinarian. Evaluation of these data did not indicate any conditions that affected the validity of the study.

E. Pretest Period

Upon arrival at DuPont Haskell, all mice were housed in quarantine. The mice were:

- quarantined for at least 6 days.
- identified temporarily by cage identification.
- weighed at least 3 times during quarantine and once prior to dosing.
- observed with respect to weight gain and any gross signs of disease or injury.

The animals were released from quarantine by the laboratory animal veterinarian or designee based on body weights and clinical signs.

F. Assignment to Groups

Animals were selected for use on study based on adequate body weight gain and freedom from any clinical signs of disease or injury. The weight variation of selected animals by sex was less than 4% of the mean weight.

Each animal was assigned an animal number and a cage identification number. The animal number and cage identification number were both included on the cage label.

At study start, the animals were at least 8 weeks old.

G. Dose Preparation, Analysis, and Rates

The test substance was prepared for administration by oral gavage. This route was chosen because it is most commonly used for toxicity studies with H-28548.

H-28548 was weighed into a vial (approximately 35.6 mg) and mixed with deionized water (100 mL). The dose solution was prepared at a nominal concentration of 0.3 mg H-28548/mL (adjusted for purity, 84%), with a target dose level of 3 mg/kg body weight (bw) and a dose volume of 10 mL/kg bw. The dose level was chosen based on the results of the 28-day daily oral

dosing study in mice, where the no-observed-adverse-effect level (NOAEL) was 0.1 and 3 mg/kg/day for males and females, respectively.⁽⁵⁾

The dosing solution was prepared prior to the day of use and was stored refrigerated at 1-10°C prior to dosing.

H. In-Life Phase

1. Material Balance and Tissue Distribution

The conduct of this study was designed to comply with the Tier 1 requirements of U.S. EPA, OPPTS 870.7485 - Metabolism and Pharmacokinetics, Health Effects Test Guidelines (1998).

Mice were housed individually in metabolism units and fasted for approximately 3 hours prior to dosing. Food was returned approximately 2 hours post-dose.

Five male and 5 female mice were administered H-28548 at a nominal target of 3 mg H-28548/kg bw. Two male and 2 female mice were each administered dose vehicle (deionized water at 10 mL/kg bw) for collection of control excreta and tissue samples. Mice were returned to individual metabolism units following dosing.

Urine and feces were collected on dry ice predose and at 0-6 h, 6-12 h, 12-24 h, and every 24 hours until 168 hours post dose. Evidence supporting a lack of metabolism of H-28548 in rat hepatocytes and rat oral dose administration studies, precluded the necessity for a radiolabeled form of H-28548 and collection of expired air.

At the end of the experiment (168 hours post dose), mice were killed by CO₂ asphyxiation followed by exsanguination. The following tissues (Tier 1) were collected:

- liver
- fat
- G.I. tract (and contents)
- kidney
- spleen
- whole blood
- residual carcass

After collection, these samples were stored at approximately ≤-10°C.

Over the course of the experiment, residual feed was collected into a single container and stored refrigerated at 1-10°C. Cages were rinsed with deionized water, which was collected into a single container. Cage wash was stored at room temperature and/or refrigerated at 1-10°C.

I. Quantitation of H-28548

1. Sample Receipt

The dose solution, urine, feces, and cage wash samples were received and stored at approximately -20°C by the analytical laboratory upon receipt and when not in use.

2. Sample Preparation Procedure (dose solution and urine samples)

The frozen samples were thawed to room temperature and mixed briefly before sampling. A pipette was used to transfer 25 µL of sample into an empty HPLC vial, and the sample weight was recorded to the nearest 0.0001 gram. The pipette was then used to add 975 µL of HPLC grade water, and mixed. The initial sample preparation dilution factor = 1/sample weight (g). Additional sample dilutions were performed with HPLC grade water to ensure that the sample peak area results were within the calibration curve limits. Quality control fortification samples were also prepared at low, mid and high levels in control urine, and prepared for analysis using the same procedure.

3. Sample Preparation Procedure (cage wash samples)

The frozen cage wash samples were thawed to room temperature and mixed briefly before sampling. A pipette was used to transfer 200 µL of sample into an empty HPLC vial, and the sample weight was recorded to the nearest 0.0001 gram. The pipette was then used to add 800 µL of HPLC grade water, and mixed. The initial sample preparation factor = 1/sample weight (g).

4. Sample Preparation Procedure (feces samples)

The frozen feces samples submitted in 15-mL conical polypropylene centrifuge tubes were thawed to room temperature. HPLC grade water was added to the 13-mL mark, and the weight of water added was recorded to the nearest 0.01 gram. Five ball bearings (5/32" diameter) were added to the sample tubes and sealed. The samples were homogenized using a Genogrinder for 5 minutes at 1400 strokes/minute (SPEX CertiPrep Genogrinder 2000, Metuchen, New Jersey U.S.A.). After homogenization, the samples were placed in a refrigerator for overnight extraction. After overnight extraction the samples were shaken to mix and centrifuged for 10 minutes at 4150 rpm at 20°C. Approximately 1.5 mL of supernatant was added to a 1.7 mL microcentrifuge tube and further centrifuged for 15 minutes at 14,000 rpm and 20 °C. A syringe filter (PALL Acrodisc - 25 mm with 0.2 µm Nylon Membrane) was then used to filter approximately 1 mL supernatant into a HPLC vial for analysis. The preparation factor = (H₂O weight (g) + feces weight (g)) / feces weight (g). Additional sample dilutions were performed with pooled control feces extract to ensure that the sample peak area results were within the calibration curve limits. Quality control fortification samples were also prepared at low, mid and high levels using 2 grams of control feces, and prepared for analysis using the same procedure.

5. Stock Solutions and Calibration Standards

A stock solution of H-28548 was prepared in HPLC grade water. The stock solution was diluted with HPLC grade water to prepare calibration standards at 0, 2.50, 5.00, 12.5, 25.0, 62.5, 156,

6. Instrument and Conditions

| | |
|-------------------------|---------------------------|
| Ion Source: | Turbo Spray, Negative Ion |
| Temperature (TEM): | 120°C |
| Dwell | 250 msec |
| Curtain Gas Flow (CUR): | 10.0 |
| GS1: | 25 |

| | | | | | |
|------------------------|---------------|---------|-------|------|------|
| GS2: | 25 | | | | |
| IonSpray (IS) Voltage: | -4500 | | | | |
| CAD | 6.00 | | | | |
| EP | -10.0 | | | | |
| Quadrupole Resolution: | Quad. 1: Unit | | | | |
| | Quad. 3: Unit | | | | |
| MRM Settings | Q1 Mass | Q3 Mass | DP | CE | CXP |
| H-28548 | 329.0 | 285.00 | -20.0 | -6.0 | -7.0 |

7. Quantitation

The samples, calibration standards, and fortification quality control plasma samples were analyzed by LC/MS/MS. The calibration standard curve was generated by regression analysis using the chromatographic peak areas of the calibration standard solutions. The peak areas for the study samples and fortification QC samples were compared to the calibration standard curve to determine the concentration of the analyte. Any samples with peak areas above the upper calibration standard were diluted to ensure that the peak areas were within the calibration curve.

J. Identification of Metabolites

Samples of urine were pooled across animals for a given time interval where the mean percent of the administered dose (by sex) was $\geq 5\%$ (males and females: 0-6, 6-12, 12-24, 24-48, and 48-72 hours; feces extract samples were not pooled since the total mean percent of dose for each collection interval (by sex) was $< 5\%$ of the administered dose.

Samples of pooled urine (25 μL) were diluted to 500 μL with Nanopure water prior to analysis. Samples of the diluted urine (20 μL) were qualitatively screened by LC/HRMS for metabolites. Retention time and mass spectral confirmation of the parent was performed by spiking control urine with approximately 40 ppm (v/v) of the test material (H-28548) and analyzing the spiked sample using the identical method for the study samples (Method 2).

1. Liquid Chromatography/Mass Spectrometry (LC/MS)

| | |
|-----------------|---|
| Method 2 | Qualitative LC/MS Confirmation and Structural Elucidation of metabolites in urine |
| HPLC/MS System: | Agilent 1100 HPLC with column thermostat and binary pump, autosampler, variable wavelength detector (S/N DE63058654 - Agilent Inc., Little Falls, Delaware, U.S.A.). Thermo-Fisher Orbitrap FT-MS (S/N 1016B - Thermo-Fisher Scientific Inc., San Jose, California, U.S.A.). The associated computer is loaded with Thermo-Fisher Xcaliber Software (v 2.0.7) |

HPLC Conditions:

| | |
|---------------------|---|
| Column: | Agilent Zorbax SB-C18 column (2.1 x 150 mm) 3.5 μm particle size |
| Column Temperature: | 25°C |
| Solvent A: | 0.10% Acetic Acid in HPLC grade water |

| | | | |
|------------|-----------------------------------|------|-------|
| Solvent B: | 0.10% Acetic acid in acetonitrile | | |
| Gradient: | Time | A | B |
| | (min) | (%) | (%) |
| | 0.0 | 98.0 | 2.0 |
| | 20.00 | 0.0 | 100.0 |
| | 25.00 | 0.0 | 100.0 |
| | 25.10 | 98.0 | 2.0 |
| | 30.00 | 98.0 | 2.0 |

Flow Rate: 0.30 mL/min
Run Time: 30.00 min
Injection Volume: 20 µL
UV Wavelength: 190-400 nm

MS Conditions:

Ionization Mode: Electrospray negative ion
Source Voltage: 3.6 kV
Capillary Temperature: 330°C
Tube Lens voltage: 140 V
Source Current: 100 µA
Data Acquisition Function: Full Scan = 120-1000 Da (Profile mode), Mass Resolution = 30,000
Daughter Scans (Da)
Identity Daughters Start Mass End Mass
of
H-28548 329 90 500
Collision Energy: 25 V daughter ion scan only
Scan Time Full scan 0.95 sec/scan; Daughter ion scan 0.3 sec/scan
Collision Gas and Pressure: Argon at 0.000602 mbar

2. Data processing

All chromatograms were screened for differences (chromatographic peaks) in control versus H28548-dosed urine samples using IntelliExtract™; v. 12.0.1 (ACD, Toronto, Ontario, Canada) control-sample comparison software.

STATISTICAL AND DATA ANALYSIS

Group data were represented as a mean ± SD.

The elimination half-life ($T_{1/2}$; time in hours to elimination of ≥50% of the administered dose) for H-28548 in male and female mice was estimated by interpolation of (mean) cumulative urinary excretion data from 0 to 168 hours using Origin v7.0220 (OriginLab Corporation, Northhampton, Massachusetts, USA).

RESULTS AND DISCUSSION

A. Quantitation of H-28548 by LC/MS/MS

(Tables 1-2, Figures 1-3)

1. Calibration Standard Curve

A calibration curve for H-28548 is shown in Figure 1. The curve was generated based on resulting peak areas of the H-28548 analyte using a quadratic equation, and 1/x weighing.

2. Limit of Detection and Limit of Quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) were determined by comparing the peak-to-peak noise in chromatograms of control matrix versus the signal of the lowest level calibration standard. The initial LOD was calculated as 3 times the concentration equivalent of the mean noise level. The initial LOQ was based on the lowest calibration standard concentration, which had at least a 10x signal-to-noise ratio. For a sample preparation factor of 1x the initial urine and cage wash sample LOD was 0.1 ng/g and for feces the initial LOD was 0.08 ng/g. For a sample preparation factor of 1x the urine, cage wash, and feces matrices all have an initial LOQ of 2.5 ng/g. The final LOD and LOQ for each sample was determined by multiplying the initial values by the sample preparation factor.

Example LOD & LOQ Calculation: Urine sample from animal 001M, 120 hour time point

- 25 μ L aliquot sample weight (g) = 0.0294 g
- Sample Preparation Factor = $1 / 0.0294 = 34.0$
- Final LOD for this sample = $0.1 \text{ ng/g} \times 34.0 = 3 \text{ ng/g}$ (reported to 1 significant digit)
- Final LOQ for this sample = $2.5 \text{ ng/g} \times 34.0 = 85.0 \text{ ng/g}$ (reported to 3 significant digits)

Example LOD & LOQ Calculation: Feces sample from animal 001M, 120 hour time point

- Water Extraction Weight = 9.89 g. Feces weight = 2.869 grams
- Sample Preparation Factor = $(9.89(\text{g}) + 2.869 (\text{g})) / 2.869 (\text{g}) = 4.45$
- Final LOD for this sample = $0.08 \text{ ng/g} \times 4.45 = 0.4 \text{ ng/g}$ (reported to 1 significant digit)
- Final LOQ for this sample = $2.5 \text{ ng/g} \times 4.45 = 11.1 \text{ ng/g}$ (reported to 3 significant digits)

Example LOD & LOQ Calculation: Cage wash sample from animal 001M, 168 hour time point

- 200 μ L aliquot sample weight (g) = 0.2097 g
- Sample Preparation Factor = $1 / 0.2097 = 4.77$

- Final LOD for this sample = $0.1 \text{ ng/g} \times 4.77 = 0.5 \text{ ng/g}$ (reported to 1 significant digit)
- Final LOQ for this sample = $2.5 \text{ ng/g} \times 4.77 = 11.9 \text{ ng/g}$ (reported to 3 significant digits)

None of the predose urine or feces samples had detectable levels of H-28548.

3. Chromatographic Results (urine, cage wash, and dose samples)

H-28548 eluted as a well-resolved peak with a retention time of approximately 2.4 minutes. An example chromatogram for the lowest calibration standard at 2.5 ng/mL is shown in Figure 2a. An example chromatogram of a urine control matrix sample is shown in Figure 2b (H-28548 was not detected). A low level fortification quality control (QC) sample is shown in Figure 2c, which was fortified at a level of 400 ng/g, and had a preparation factor of 40x. A 24-hour urine sample from animal 001M, which had a total dilution factor of 1480x is shown in Figure 2d. The final concentration for this sample was 14,800 ng/g.

4. Chromatographic Results (feces samples)

H-28548 eluted as a well-resolved peak with a retention time of approximately 5 minutes. An example chromatogram for the lowest calibration standard at 2.5 ng/mL is shown in Figure 3a. An example chromatogram of a feces control matrix sample is shown in Figure 3b (H-28548 was not detected). A low level fortification quality control (QC) sample is shown in Figure 3c, which was fortified at a level of 250 ng/g, and had a preparation factor of 6.14x. A 12 hour feces sample from animal 001M, which had a total dilution factor of 34.0x is shown in Figure 3d. The final concentration for this sample was 775 ng/g.

5. Fortification QC Sample Results

The average QC fortification results for the urine matrix are provided in Table 1. The average recoveries for the low level, mid level, and high level fortification standards ranged from 101-102%. The associated coefficient of variation (CV) was 1% for each level and demonstrates acceptable method performance.

The average QC fortification results for the feces matrix are provided in Table 2. The average recoveries for the low level, mid level, and high level fortification standards ranged from 88-100%. The associated CV ranged from 2-4% and demonstrates acceptable method performance.

B. Dose Formulation Concentration, Animal Body Weights, Dosing Information

(Table 3, Appendices A-B)

The concentration of H-28548 in the dose solution, as confirmed by LC/MS, was 0.29 mg H-28548/mL, which was >96% of the nominal target (0.3 mg H-28548/mL).

At study initiation (day of dosing), males weighed $27.0 \text{ g} \pm 0.47 \text{ g}$ and females weighed $24.5 \text{ g} \pm 0.52 \text{ g}$; the calculated dose rate for male ($2.91 \pm 0.03 \text{ mg/kg bw}$) and female mice ($2.91 \pm 0.06 \text{ mg/kg bw}$) were within 3% of the nominal target (3 mg/kg bw).

C. Urine Data

(Table 4, Figure 4, Appendix C)

Following oral administration of H-28548 in water, $30.8\% \pm 5.37\%$ and $39.3\% \pm 5.58\%$ of the administered dose (0-12 hours) was accounted for in urine from male and female mice, respectively.

At the conclusion of the study (168 hours post-dose), the cumulative amount of H-28548 detected in urine was $89.5\% \pm 6.91\%$ and $91.5\% \pm 6.04\%$ for male and female mice, respectively.

Elimination of H-28548 via urine accounted for the administered dose for both male and female mice.

D. Feces Data

(Table 5, Figure 5, Appendix D)

Following oral administration of H-28548 in water, the cumulative amount of H-28548 detected in feces over the entire collection period (0-168 hours) was $2.00\% \pm 1.01\%$ and $1.91\% \pm 0.85\%$ for male and female mice, respectively.

The minor amount of H-28548 detected in feces was likely contamination from urine. Given the high levels of H-28548 in urine, and the design of the urine/feces collection system of the metabolism units, feces likely became contaminated with small amounts of urine when contacting surfaces in transit to the feces collection vessel.

E. Material Balance

(Table 6, Figure 6, Appendices E-F)

Following oral dosing with H-28548 in water and a 168 hour post-dose collection period, $101.2\% \pm 3.22\%$ and $99.7\% \pm 2.95\%$ of the administered dose was recovered from male and female mice, respectively.

Of the total H-28548 recovered, the majority of administered dose was account for in urine from both males ($89.5\% \pm 6.91\%$) and females ($91.5\% \pm 6.04\%$); lesser amounts of H-28548 were accounted for in feces (male = $2.00\% \pm 1.01\%$; female = $1.91\% \pm 0.85\%$). Cagewash, which is composed of dried excreta (urine and feces) accounted for $9.64\% \pm 3.99\%$ and $6.25\% \pm 3.16\%$ of the administered dose for male and female mice, respectively.

The carcass and residual feed were not analyzed for H-28548 because analysis of urine, feces and cagewash accounted for the majority of administered dose with an overall recovery of $100\% \pm 10\%$.

F. Metabolite Identification

(Figures 7-9)

H-28548 was detected in its anionic form by negative ESI mass spectrometry. A representative reconstructed chromatogram of ions characteristic of H-28548 (parent) for the 6 hour female dosed mouse urine sample and control urine fortified with the H-28548 test substance is shown in Figure 7.

The LC/MS mass spectrum of H-28548 in urine shows a significant amount of its proton bound dimer (m/z 658.943 Da) and sodium bound dimer (m/z 680.923 Da) (Figure 8); the dimer and the sodium dimer were created in the MS system and were not present in the sample itself. The molecular anion (m/z 328.968) was observed in both urine from a mouse dosed with H-28548 and the urine fortified with the test substance H-28548, but at a low intensity relative to the dimer adducts. These dimers are not to be confused with a covalent dimer, such as the HFPO acid dimer parent, but are charged dimers sometimes formed, in-source, as a result of the desolvation and ionization processes necessary to be observed by electrospray ionization mass spectrometry.

The daughter ion mass spectra of the parent ion 328.97 Da for urine from a mouse dosed with H-28548 and urine fortified with the H-28548 test substance shows the same 2 characteristic fragment ions at m/z 284.977, the loss of CO_2 and 169.989, $[\text{C}_3\text{F}_7]^-$ (Figure 9).

Subsequent to collection of the LC/MS, all sample data were screened for suspected metabolites manually and automatically for unexpected metabolites using the IntelliExtractTM control-comparison data processing tool. In all cases, there was no evidence of metabolism observed in any of the samples by either method and only the anionic form of the residual parent, H-28548, was detected.

G. Elimination Half-Life ($T_{1/2}$)

(Appendix G)

The elimination half-life ($T_{1/2}$) for H-28548 in male and female mice, following a single oral dose at 3 mg/kg, was estimated to be 21 and 18 hours, respectively.

CONCLUSIONS

Following oral administration of H-28548 in water, $30.8\% \pm 5.37\%$ and $39.3\% \pm 5.58\%$ of the administered dose was accounted for in urine (0-12 hours) from male and female mice, respectively. At the conclusion of the study (168 hours post-dose), the total accumulated amount of H-28548 detected in urine was $89.5\% \pm 6.91\%$ and $91.5\% \pm 6.04\%$ of the administered dose for male and female mice, respectively.

Elimination of H-28548 via urine accounted for a majority of the administered dose for both male and female mice; minor levels of H-28548 detected in feces from male ($2.00\% \pm 1.01\%$) and female mice ($1.91\% \pm 0.85\%$) were likely contamination from of urine.

Cagewash, which is composed of dried excreta (urine and feces), accounted for $9.64\% \pm 3.99\%$ and $6.25\% \pm 3.16\%$ of the administered dose for male and female mice, respectively.

Following oral dosing with H-28548 in water and a 168 hour post-dose collection period, $101.2\% \pm 3.22\%$ and $99.7\% \pm 2.95\%$ of the administered dose was recovered from male and female mice, respectively.

Samples of urine evaluated using LC/MS were found to contain only the parent substance, H-28548. This finding, taken with recovery of the administered dose in urine, confirms that H-28548 was rapidly absorbed and eliminated unmetabolized following oral dosing in the mouse.

The elimination half-life ($T_{1/2}$) for H-28548 in male and female mice, following a single oral dose at 3 mg/kg, was estimated to be 21 and 18 hours, respectively.

RECORDS AND SAMPLE STORAGE

Specimens (if applicable), raw data, the protocol, amendments (if any), and the final report will be retained at DuPont Haskell, Newark, Delaware, Iron Mountain Records Management, Wilmington, Delaware, or Quality Associates Incorporated, Fulton, Maryland.

REFERENCES

1. DuPont Haskell (2007). In Vitro Rat Hepatocyte Screen. Unpublished report, DuPont-23460.
2. DuPont Haskell (2008). Repeated Dose Oral Toxicity 7-Day Gavage Study in Rats. Unpublished report, DuPont-24009.
3. DuPont Haskell (2007). Biopersistence and Pharmacokinetic Screen in Rats. Unpublished report, DuPont-24281.
4. DuPont Haskell (2009). Cross-Species Comparison of FRD-902 Plasma Pharmacokinetics in the Rat and Primate Following Intravenous Dosing. Unpublished report, DuPont-17751-1579 RV1.
5. DuPont-Haskell (2008). A 28-Day Oral (Gavage) Toxicity Study of H-28397 in Rats with a 28-Day Recovery. Unpublished report, DuPont-24447.

TABLES

TABLES

EXPLANATORY NOTES

ABBREVIATIONS:

CV - coefficient of variation
NA - not applicable
QC - quality control
SD - standard deviation

Table 1
Mouse urine sample fortification QC results for H-28548

| Mouse Urine Fortification Sample | Fortification Concentration (ng/g) | Average Recovery (%) | CV (%) |
|--|--|----------------------------|-----------|
| Low | 400 | 102 | 1 |
| Mid | 100,000 | 100 | 1 |
| High | 1,000,000 | 101 | 1 |

Table 2
Mouse feces sample fortification QC result for H-28548

| Mouse Feces Fortification Sample | Fortification Concentration (ng/g) | Average Recovery (%) | CV (%) |
|--|--|----------------------------|-----------|
| Low | 250 | 88 | 4 |
| Mid | 1250 | 95 | 4 |
| High | 50,000 | 100 | 2 |

Table 3
Dosing information

| | Males | | Females | |
|------------------------------|-------|-------|---------|-------|
| | Mean | SD | Mean | SD |
| Subject weight (g) | 27.0 | 0.47 | 24.5 | 0.52 |
| Test substance received (mg) | 0.079 | 0.001 | 0.071 | 0.002 |
| Dose (mg/kg bw) | 2.91 | 0.03 | 2.91 | 0.06 |

Table 4
Urine, cumulative percent of dose

| Post-Dose Time Point (hours) | Males | | Females | |
|---------------------------------|-------|------|---------|------|
| | Mean | SD | Mean | SD |
| Pre-dose | NA | NA | NA | NA |
| 6 | 14.1 | 5.19 | 17.2 | 4.41 |
| 12 | 30.8 | 5.37 | 39.3 | 5.58 |
| 24 | 54.9 | 6.26 | 61.4 | 5.99 |
| 48 | 72.7 | 8.10 | 77.9 | 5.58 |
| 72 | 80.0 | 7.22 | 84.3 | 6.64 |
| 96 | 84.1 | 7.12 | 87.7 | 6.67 |
| 120 | 86.5 | 7.16 | 89.5 | 6.55 |
| 144 | 88.2 | 7.14 | 90.7 | 6.22 |
| 168 | 89.5 | 6.91 | 91.5 | 6.04 |

Table 5
Feces, cumulative percent of dose

| Post-Dose Time Point (hours) | Males | | Females | |
|---------------------------------|-------|------|---------|------|
| | Mean | SD | Mean | SD |
| 0 | NA | NA | NA | NA |
| 6 | 0.31 | 0.4 | NA | NA |
| 12 | 0.56 | 0.38 | 0.70 | 0.46 |
| 24 | 0.86 | 0.37 | 0.89 | 0.55 |
| 48 | 1.34 | 0.56 | 1.40 | 0.47 |
| 72 | 1.54 | 0.63 | 1.63 | 0.58 |
| 96 | 1.71 | 0.73 | 1.70 | 0.60 |
| 120 | 1.80 | 0.78 | 1.80 | 0.71 |
| 144 | 1.89 | 0.89 | 1.89 | 0.82 |
| 168 | 2.00 | 1.01 | 1.91 | 0.85 |

Table 6
Material balance, percent of dose

| | Males | | Females | |
|-----------|-------|------|---------|------|
| | Mean | SD | Mean | SD |
| Urine | 89.5 | 6.91 | 91.5 | 6.04 |
| Feces | 2.00 | 1.01 | 1.91 | 0.85 |
| Cage Wash | 9.64 | 3.99 | 6.25 | 3.16 |
| Total | 101.2 | 3.22 | 99.7 | 2.95 |

FIGURES

FIGURES

EXPLANATORY NOTES

ABBREVIATIONS:

QC - quality control
cps - counts per second
m/z - mass-to-charge ratio
min - minute

Figure 1
Calibration curve for H-28548

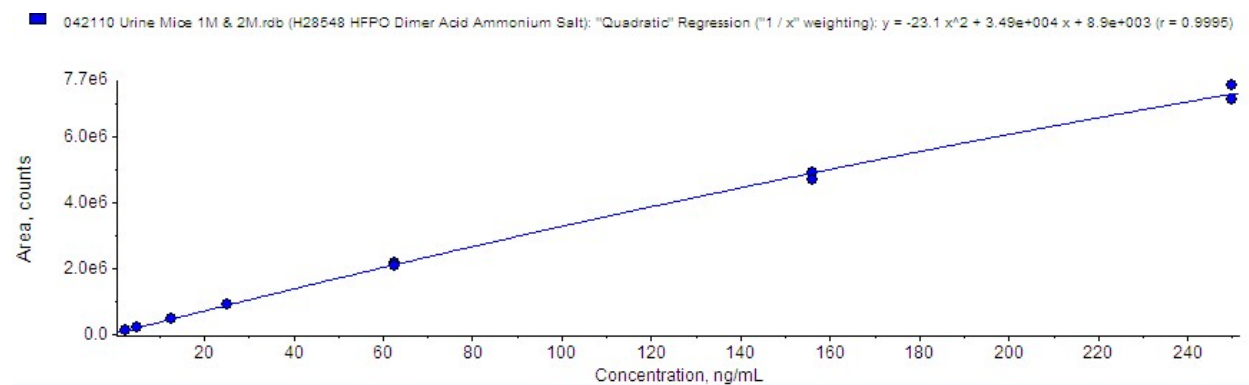


Figure 2

The LC/MS/MS chromatograms for a) lowest calibration standard at 2.5 ng/mL, b) urine control matrix sample, c) low level 400 ng/g fortification QC sample with preparation factor 40x, and d) a 24-hour urine study sample from animal 001M, which had a total dilution factor of 1480x and final concentration of 14,800 ng/g

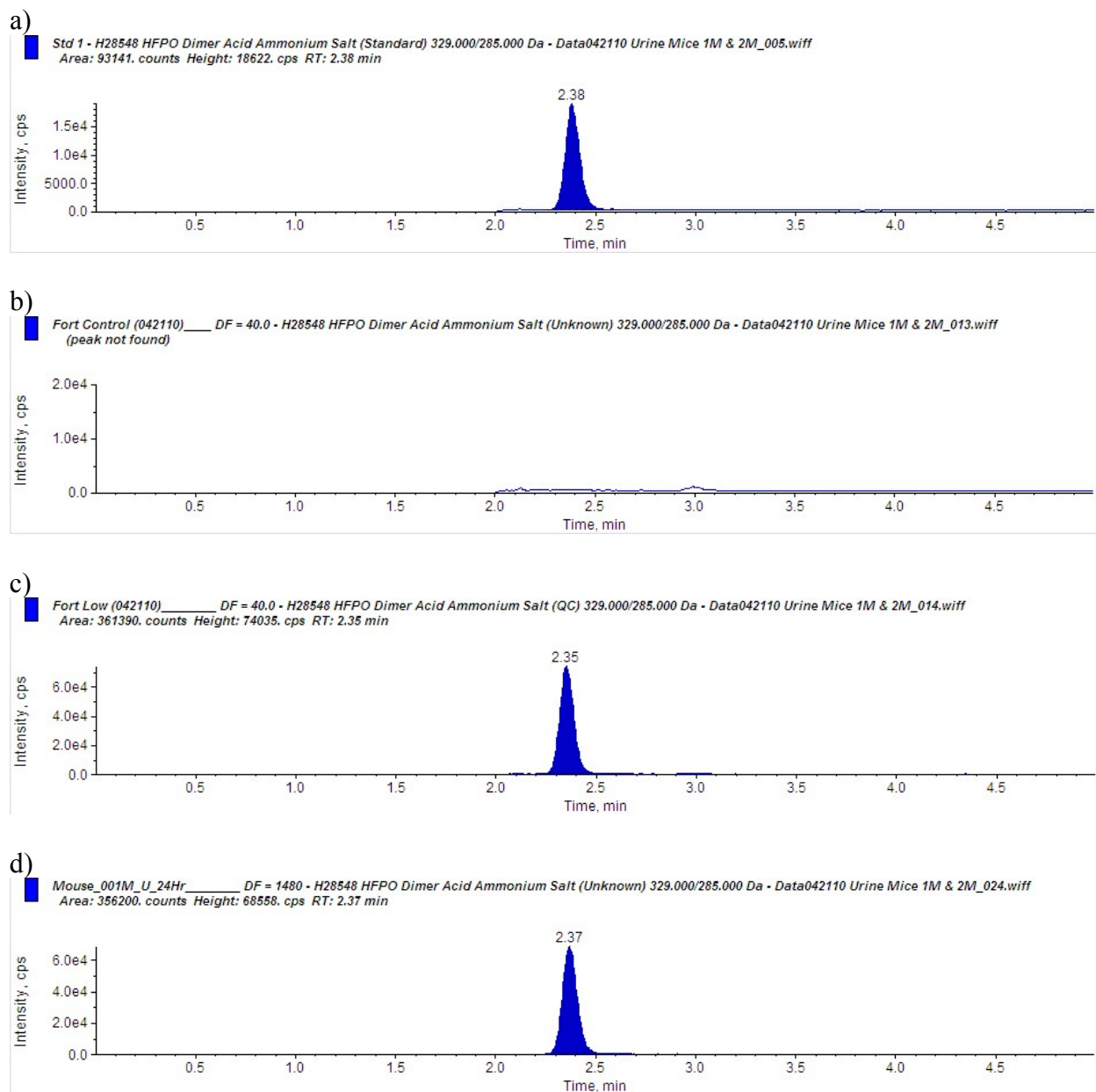


Figure 3

The LC/MS/MS chromatograms for a) lowest calibration standard at 2.5 ng/mL, b) feces control matrix sample, c) low level 250 ng/g fortification QC sample that had a preparation factor of 6.14x, and d) a 12-hour feces study sample from animal 001M, which had a 34x dilution factor and final concentration of 775 ng/g

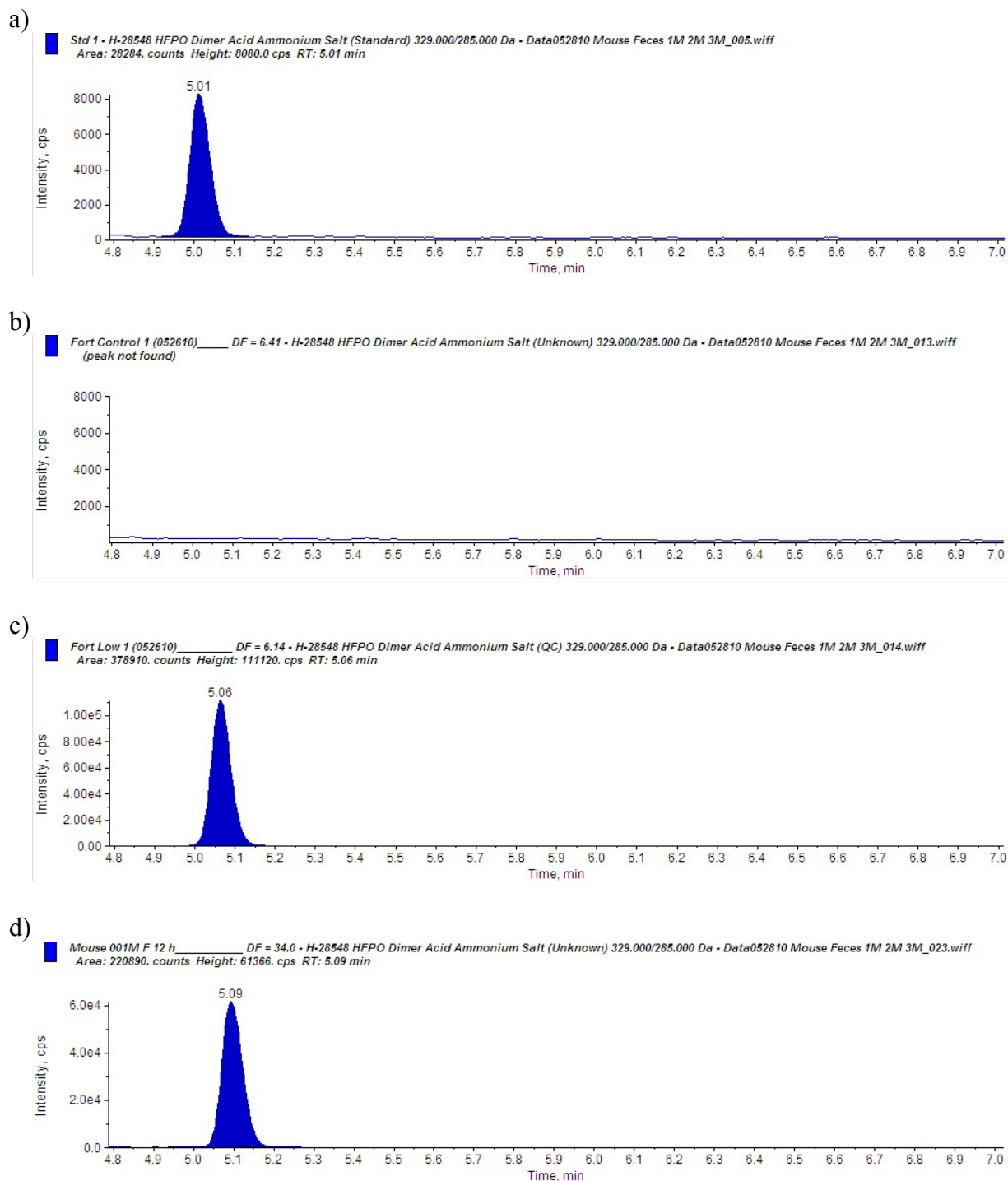


Figure 4
Urine, cumulative percent

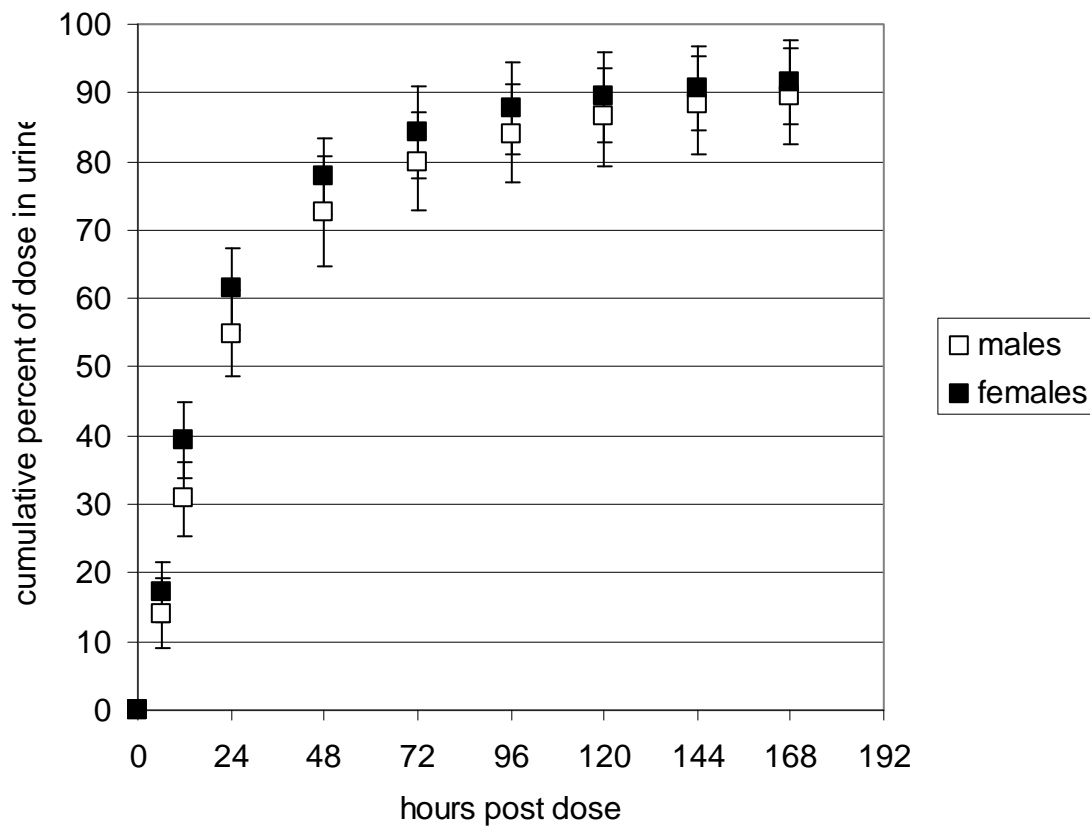


Figure 5
Feces, cumulative percent

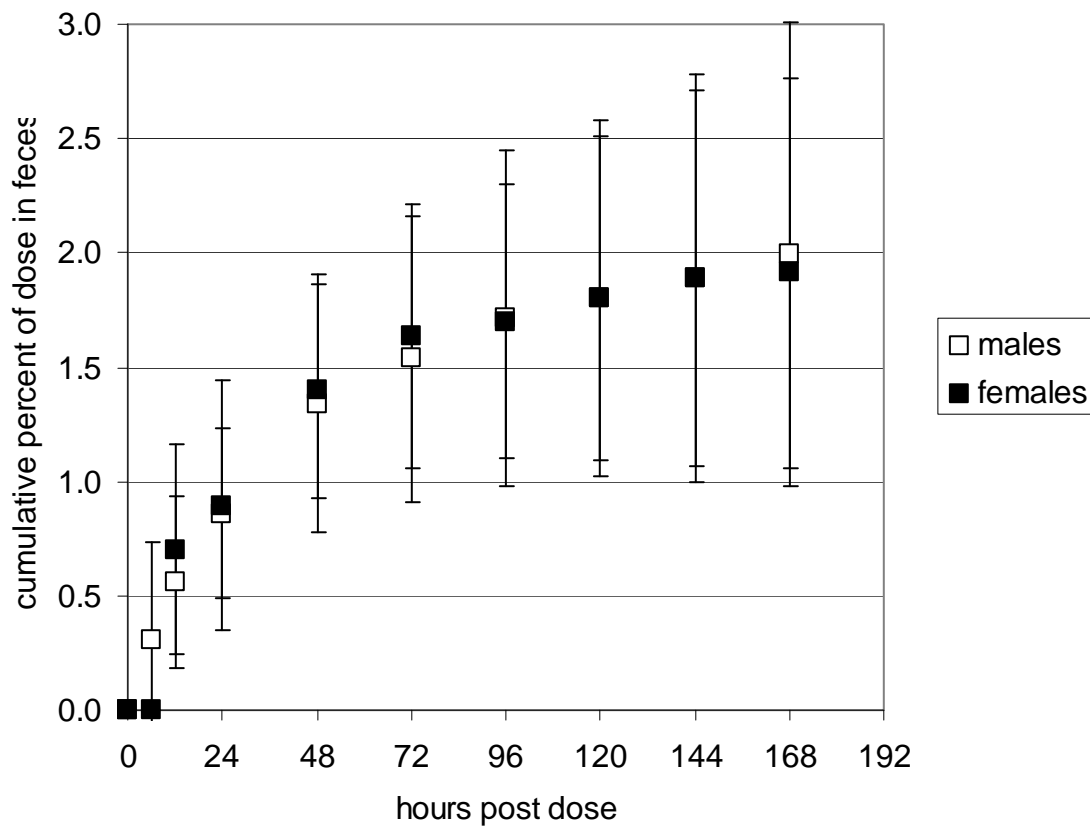


Figure 6
Material Balance, percent of dose

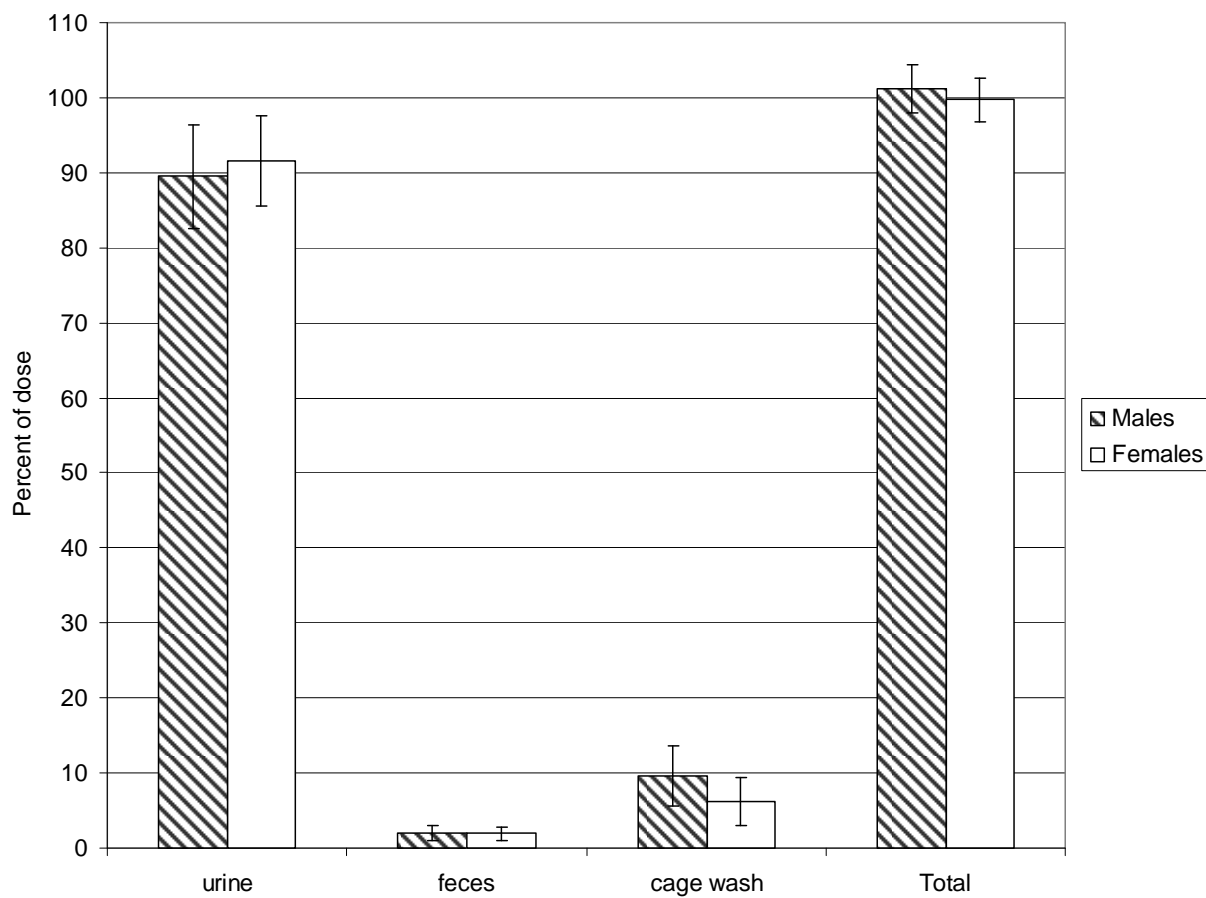


Figure 7
Reconstructed m/z 329 + 659 ion chromatograms characteristic of H-28548-dosed female mouse urine (6 hours after administration) – top and control mouse urine fortified with H-28458 test substance -bottom

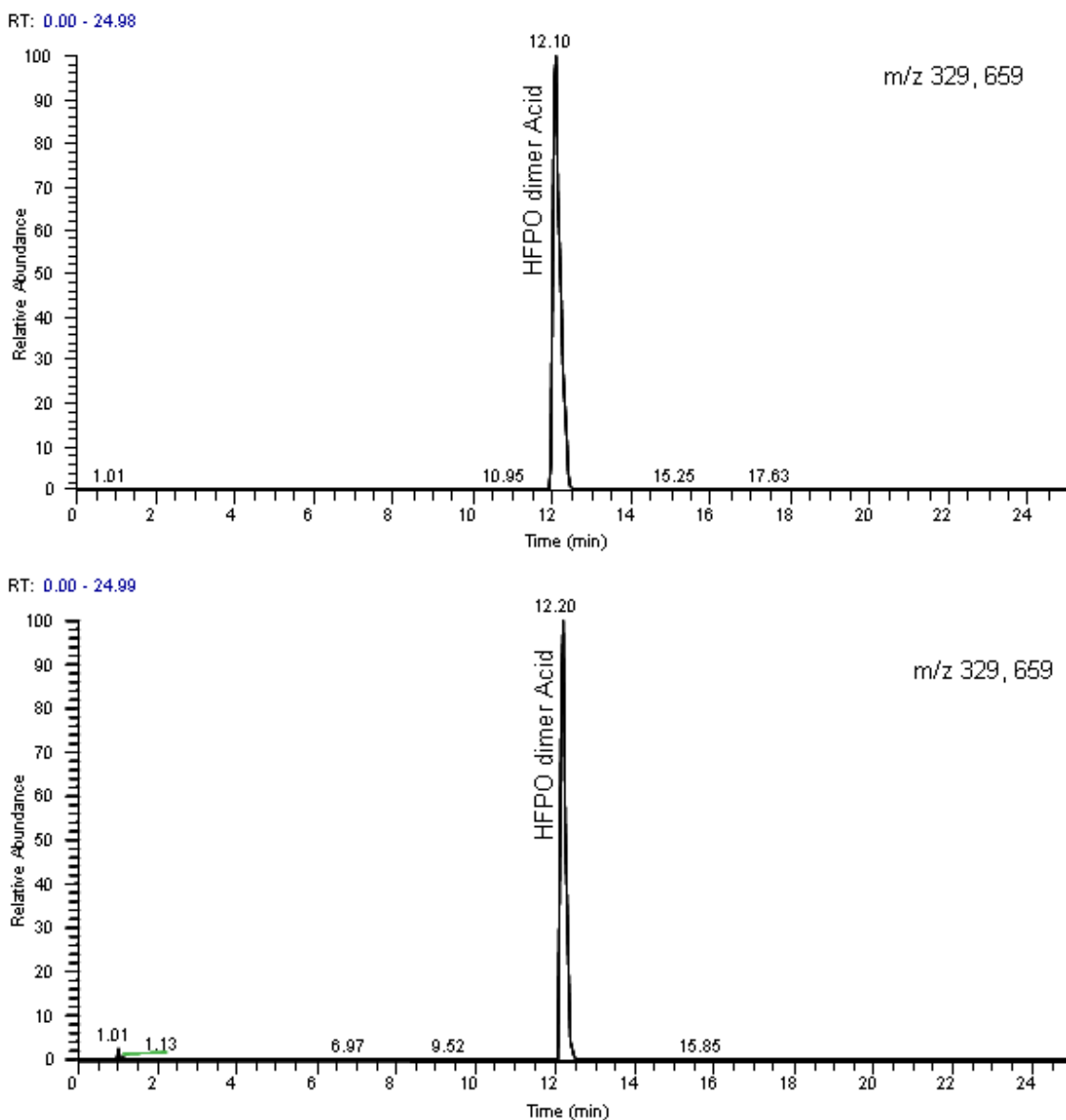


Figure 8
ESI negative mass spectra of H-28548 observed in dosed female mouse urine (6 hours after administration)–top; and control urine fortified with H-28548 test substance – bottom

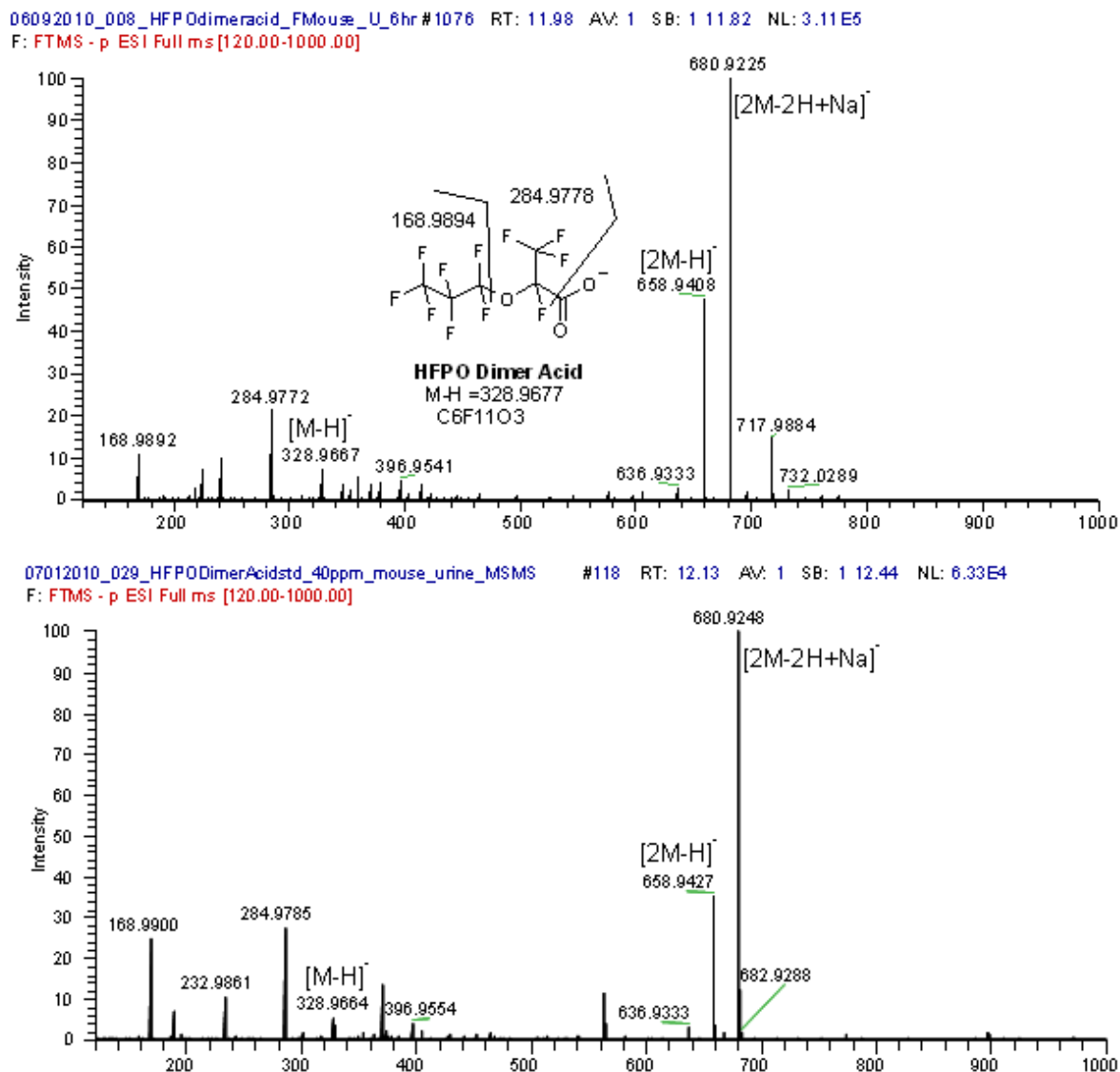
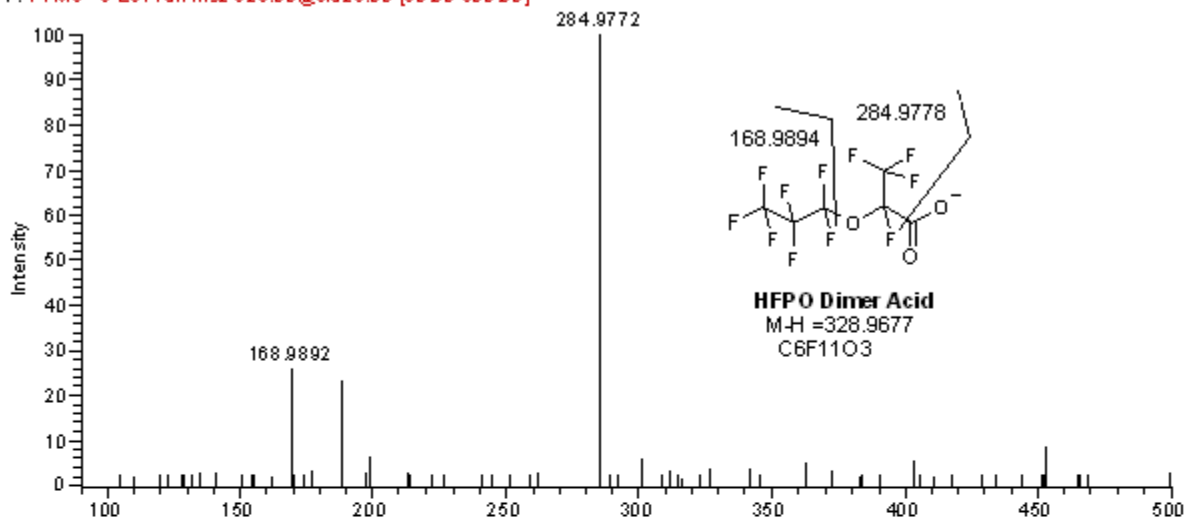


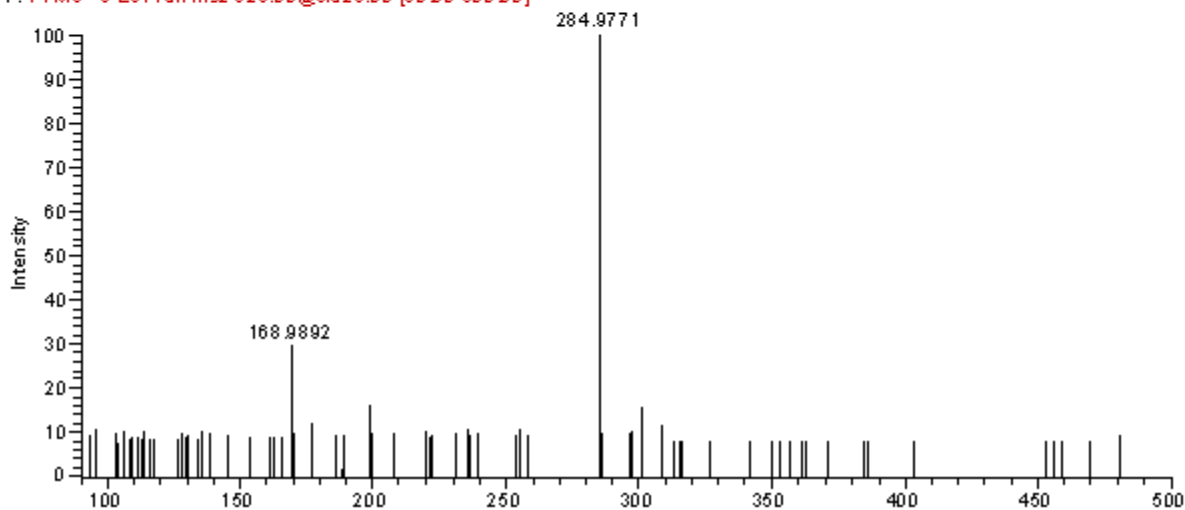
Figure 9

ESI negative daughter ion mass spectra of H28548 observed in dosed female mouse urine (6 hours after administration)–top; and control mouse urine fortified with H-28548 test substance – bottom

07012010_005B_HFPO Dimer Acid FMouse_U_6hr_MSMS #575 RT: 12.11 AV: 1 SB: 1 12.48 NL: 9.64E3
F: FTMS - c ESI Full ms2 329.00@cid25.00 [90.00-500.00]



07012010_006A_HFPO Dimer Acid std_40ppm_mouse_urine_MSMS #566 RT: 12.15 AV: 1 SB: 1 9.79 NL: 2.43E3
F: FTMS - c ESI Full ms2 329.00@cid25.00 [90.00-500.00]



APPENDICES

APPENDICES

EXPLANATORY NOTES

ABBREVIATIONS:

F - female
h - hours
LOQ - limit of quantification
M - male
NA - not applicable
ND - not detected
SD - standard deviation

Appendix A
Certificate of Analysis




E. I. du Pont de Nemours and Company
Wilmington, DE 19898
USA

CERTIFICATE OF ANALYSIS

This Certificate of Analysis fulfills the requirement for characterization of a test substance prior to a study subject to GLP regulations. It documents the identity and content of the test substance. This work was conducted under EPA Good Laboratory Practice Standards (40 CFR 792).

| | |
|--------------------------|---|
| Haskell Code Number | H-28548 |
| Common Name | HFPO Dimer Acid Ammonium Salt |
| Purity Percent | 84% |
| Other Components | Water – 12.7% Perfluorooctanoic acid – 150 ppm |
| Date of Analysis | June 13, 2008 |
| Expiration Date | June 13, 2011 |
| Instructions for storage | NRT&H |
| Reference | DuPont-25455 |
| Analysis performed at | E. I. DuPont de Nemours and Company DuPont Haskell Laboratories Newark, Delaware USA |

Approver:


Peter A. Bloxham, Ph.D.
Senior Research Chemist

24-JUN-2009
Date

Revision #1: Revised COA expiration date based on compound stability assessment. 6/23/09

Appendix B

Dosing Information

Dosing Information

| Males Subject | Subject weight (g) | Compound received (mg) | Dose rate (mg/kg) |
|------------------|--------------------------|------------------------------|-------------------------|
| 001M | 27.7 | 0.081 | 2.91 |
| 002M | 27.2 | 0.079 | 2.90 |
| 003M | 26.8 | 0.077 | 2.89 |
| 004M | 26.6 | 0.077 | 2.90 |
| 005M | 26.6 | 0.079 | 2.95 |
| Mean | 27.0 | 0.079 | 2.91 |
| SD | 0.47 | 0.001 | 0.03 |

| Females Subject | Subject weight (g) | Compound received (mg) | Dose rate (mg/kg) |
|--------------------|--------------------------|------------------------------|-------------------------|
| 001F | 24.3 | 0.073 | 3.01 |
| 002F | 24.2 | 0.070 | 2.88 |
| 003F | 24.5 | 0.070 | 2.85 |
| 004F | 24.1 | 0.071 | 2.94 |
| 005F | 25.4 | 0.073 | 2.89 |
| Mean | 24.5 | 0.071 | 2.91 |
| SD | 0.52 | 0.002 | 0.06 |

Appendix C
Urine Data

Urine Data - Males

| Animal Number | Total H-28548 (ng) | Timepoint (hours) | Sample weight (g) | Concentration H-28548 (ng/g) | Total Amount (ng H-28548) | Percent | Cumulative (%) |
|---------------|--------------------|-------------------|-------------------|------------------------------|---------------------------|---------|----------------|
| 001M | 80620 | Pre-dose | 1.23 | ND | NA | NA | NA |
| | | 6 h | 0.467 | 36000 | 16812 | 20.9 | 20.9 |
| | | 12 h | 0.24 | 31300 | 7512 | 9.32 | 30.2 |
| | | 24 h | 1.608 | 14800 | 23798 | 29.5 | 59.7 |
| | | 48 h | 1.672 | 8060 | 13476 | 16.7 | 76.4 |
| | | 72 h | 1.794 | 2780 | 4987 | 6.19 | 82.6 |
| | | 96 h | 1.907 | 1720 | 3280 | 4.07 | 86.7 |
| | | 120 h | 1.795 | 1070 | 1921 | 2.38 | 89.0 |
| | | 144 h | 1.58 | 722 | 1141 | 1.41 | 90.5 |
| | | 168 h | 1.798 | 537 | 966 | 1.20 | 91.7 |
| | | | | | | 91.7 | |

| Animal Number | Total H-28548 (ng) | Timepoint (hours) | Sample weight (g) | Concentration H-28548 (ng/g) | Total Amount (ng H-28548) | Percent | Cumulative (%) |
|---------------|--------------------|-------------------|-------------------|------------------------------|---------------------------|---------|----------------|
| 002M | 78880 | Pre-dose | 2.155 | ND | NA | NA | NA |
| | | 6 h | 0.599 | 24300 | 14556 | 18.5 | 18.5 |
| | | 12 h | 0.311 | 32900 | 10232 | 13.0 | 31.4 |
| | | 24 h | 1.403 | 12000 | 16836 | 21.3 | 52.8 |
| | | 48 h | 2.12 | 6600 | 13992 | 17.7 | 70.5 |
| | | 72 h | 2.489 | 2720 | 6770 | 8.58 | 79.1 |
| | | 96 h | 2.833 | 1240 | 3513 | 4.45 | 83.5 |
| | | 120 h | 2.866 | 755 | 2164 | 2.74 | 86.3 |
| | | 144 h | 2.595 | 506 | 1313 | 1.66 | 88.0 |
| | | 168 h | 3.139 | 326 | 1023 | 1.30 | 89.2 |
| | | | | | | 89.2 | |

| Animal Number | Total H-28548 (ng) | Timepoint (hours) | Sample weight (g) | Concentration H-28548 (ng/g) | Total Amount (ng H-28548) | Percent | Cumulative (%) |
|---------------|--------------------|-------------------|-------------------|------------------------------|---------------------------|---------|----------------|
| 003M | 77430 | Pre-dose | 1.441 | ND | NA | NA | NA |
| | | 6 h | 0.312 | 29100 | 9079 | 11.7 | 11.7 |
| | | 12 h | 0.576 | 27300 | 15725 | 20.3 | 32.0 |
| | | 24 h | 0.95 | 18200 | 17290 | 22.3 | 54.4 |
| | | 48 h | 1.656 | 11500 | 19044 | 24.6 | 79.0 |
| | | 72 h | 1.275 | 3940 | 5024 | 6.49 | 85.4 |
| | | 96 h | 1.375 | 2460 | 3383 | 4.37 | 89.8 |
| | | 120 h | 1.191 | 1600 | 1906 | 2.46 | 92.3 |
| | | 144 h | 1.334 | 1020 | 1361 | 1.76 | 94.0 |
| | | 168 h | 1.469 | 669 | 983 | 1.27 | 95.3 |
| | | | | | | 95.3 | |

Urine Data - Males

| Animal Number | Total H-28548 (ng) | Timepoint (hours) | Sample weight (g) | Concentration H-28548 (ng/g) | Total Amount (ng H-28548) | Percent | Cumulative (%) |
|------------------|--------------------------|----------------------|----------------------|------------------------------------|---------------------------------|---------|-------------------|
| 004M | 77140 | Pre-dose | 1.291 | ND | NA | NA | NA |
| | | 6 h | 0.27 | 26300 | 7101 | 9.2 | 9.21 |
| | | 12 h | 0.227 | 45800 | 10397 | 13.5 | 22.7 |
| | | 24 h | 0.897 | 19900 | 17850 | 23.1 | 45.8 |
| | | 48 h | 0.917 | 11500 | 10546 | 13.7 | 59.5 |
| | | 72 h | 1.385 | 4650 | 6440 | 8.35 | 67.8 |
| | | 96 h | 1.315 | 2470 | 3248 | 4.21 | 72.1 |
| | | 120 h | 0.803 | 2170 | 1743 | 2.26 | 74.3 |
| | | 144 h | 1.236 | 1140 | 1409 | 1.83 | 76.1 |
| | | 168 h | 1.43 | 924 | 1321 | 1.71 | 77.9 |
| | | | | | | 77.9 | |

| Animal Number | Total H-28548 (ng) | Timepoint (hours) | Sample weight (g) | Concentration H-28548 (ng/g) | Total Amount (ng H-28548) | Percent | Cumulative (%) |
|------------------|--------------------------|----------------------|----------------------|------------------------------------|------------------------------|---------|-------------------|
| 005M | 78590 | Pre-dose | 1.621 | ND | NA | NA | NA |
| | | 6 h | 0.176 | 46600 | 8202 | 10.4 | 10.4 |
| | | 12 h | 0.366 | 58500 | 21411 | 27.2 | 37.7 |
| | | 24 h | 0.883 | 21400 | 18896 | 24.0 | 61.7 |
| | | 48 h | 1.515 | 8560 | 12968 | 16.5 | 78.2 |
| | | 72 h | 1.432 | 3620 | 5184 | 6.60 | 84.8 |
| | | 96 h | 1.318 | 2100 | 2768 | 3.52 | 88.3 |
| | | 120 h | 1.76 | 1010 | 1778 | 2.26 | 90.6 |
| | | 144 h | 1.995 | 781 | 1558 | 1.98 | 92.6 |
| | | 168 h | 2.318 | 358 | 830 | 1.06 | 93.6 |
| | | | | | | 93.6 | |

| Timepoint (hours) | Cumulative Mean | SD |
|----------------------|--------------------|------|
| 0 h | NA | NA |
| 6 h | 14.1 | 5.2 |
| 12 h | 30.8 | 5.37 |
| 24 h | 54.9 | 6.26 |
| 48 h | 72.7 | 8.10 |
| 72 h | 80.0 | 7.22 |
| 96 h | 84.1 | 7.12 |
| 120 h | 86.5 | 7.16 |
| 144 h | 88.2 | 7.14 |
| 168 h | 89.5 | 6.91 |

Urine Data - Females

| Animal Number | Total H-28548 (ng) | Timepoint (hours) | Sample weight (g) | Concentration H-28548 (ng/g) | Total Amount (ng H-28548) | Percent | Cumulative (%) |
|---------------|--------------------|-------------------|-------------------|------------------------------|---------------------------|---------|----------------|
| 001F | 73080 | Pre-dose | 1.165 | ND | NA | NA | NA |
| | | 6 h | 0.318 | 28400 | 9031 | 12.4 | 12.4 |
| | | 12 h | 0.305 | 57500 | 17538 | 24.0 | 36.4 |
| | | 24 h | 0.999 | 19600 | 19580 | 26.8 | 63.1 |
| | | 48 h | 2.114 | 6260 | 13234 | 18.11 | 81.3 |
| | | 72 h | 1.355 | 2780 | 3767 | 5.15 | 86.4 |
| | | 96 h | 2.392 | 1230 | 2942 | 4.03 | 90.4 |
| | | 120 h | 2.333 | 486 | 1134 | 1.55 | 92.0 |
| | | 144 h | 2.149 | 270 | 580 | 0.79 | 92.8 |
| | | 168 h | 2.425 | 183 | 444 | 0.61 | 93.4 |
| | | | | | | 93.4 | |

| Animal Number | Total H-28548 (ng) | Timepoint (hours) | Sample weight (g) | Concentration H-28548 (ng/g) | Total Amount (ng H-28548) | Percent | Cumulative (%) |
|---------------|--------------------|-------------------|-------------------|------------------------------|---------------------------|---------|----------------|
| 002F | 69600 | Pre-dose | 1.164 | ND | NA | NA | NA |
| | | 6 h | 0.414 | 32500 | 13455 | 19.3 | 19.3 |
| | | 12 h | 0.29 | 45800 | 13282 | 19.1 | 38.4 |
| | | 24 h | 0.958 | 15600 | 14945 | 21.5 | 59.9 |
| | | 48 h | 1.856 | 6580 | 12212 | 17.5 | 77.4 |
| | | 72 h | 1.442 | 2490 | 3591 | 5.16 | 82.6 |
| | | 96 h | 1.931 | 1100 | 2124 | 3.05 | 85.6 |
| | | 120 h | 2.51 | 476 | 1195 | 1.72 | 87.4 |
| | | 144 h | 2.853 | 501 | 1429 | 2.05 | 89.4 |
| | | 168 h | 1.959 | 443 | 868 | 1.25 | 90.7 |
| | | | | | | 90.7 | |

| Animal Number | Total H-28548 (ng) | Timepoint (hours) | Sample weight (g) | Concentration H-28548 (ng/g) | Total Amount (ng H-28548) | Percent | Cumulative (%) |
|---------------|--------------------|-------------------|-------------------|------------------------------|---------------------------|---------|----------------|
| 003F | 69890 | Pre-dose | 2.575 | ND | NA | NA | NA |
| | | 6 h | 0.287 | 55400 | 15900 | 22.7 | 22.7 |
| | | 12 h | 0.41 | 45000 | 18450 | 26.4 | 49.1 |
| | | 24 h | 0.873 | 17300 | 15103 | 21.6 | 70.8 |
| | | 48 h | 1.975 | 4540 | 8967 | 12.8 | 83.6 |
| | | 72 h | 2.041 | 2580 | 5266 | 7.53 | 91.1 |
| | | 96 h | 2.368 | 736 | 1743 | 2.49 | 93.6 |
| | | 120 h | 3.804 | 334 | 1271 | 1.82 | 95.4 |
| | | 144 h | 2.232 | 231 | 516 | 0.74 | 96.2 |
| | | 168 h | 1.971 | 175 | 345 | 0.49 | 96.7 |
| | | | | | | 96.7 | |

Urine Data - Females

| Animal Number | Total H-28548 (ng) | Timepoint (hours) | Sample weight (g) | Concentration H-28548 (ng/g) | Total Amount (ng H-28548) | Percent | Cumulative (%) |
|------------------|--------------------------|----------------------|----------------------|------------------------------------|---------------------------------|---------|-------------------|
| 004F | 70760 | Pre-dose | 0.706 | ND | NA | NA | NA |
| | | 6 h | 0.481 | 27200 | 13083 | 18.5 | 18.5 |
| | | 12 h | 0.251 | 50000 | 12550 | 17.7 | 36.2 |
| | | 24 h | 0.642 | 20800 | 13354 | 18.9 | 55.1 |
| | | 48 h | 1.447 | 6760 | 9782 | 13.8 | 68.9 |
| | | 72 h | 1.384 | 2490 | 3446 | 4.87 | 73.8 |
| | | 96 h | 1.433 | 1600 | 2293 | 3.24 | 77.0 |
| | | 120 h | 1.553 | 909 | 1412 | 2.00 | 79.0 |
| | | 144 h | 1.718 | 603 | 1036 | 1.46 | 80.5 |
| | | 168 h | 1.52 | 484 | 736 | 1.04 | 81.5 |
| | | | | | | 81.5 | |

| Animal Number | Total H-28548 (ng) | Timepoint (hours) | Sample weight (g) | Concentration H-28548 (ng/g) | Total Amount (ng H-28548) | Percent | Cumulative (%) |
|------------------|--------------------------|----------------------|----------------------|------------------------------------|------------------------------|---------|-------------------|
| 005F | 73370 | Pre-dose | 1.152 | ND | NA | NA | NA |
| | | 6 h | 0.266 | 35900 | 9549 | 13.0 | 13.0 |
| | | 12 h | 0.483 | 35500 | 17147 | 23.4 | 36.4 |
| | | 24 h | 1.092 | 14600 | 15943 | 21.7 | 58.1 |
| | | 48 h | 2.702 | 5510 | 14888 | 20.3 | 78.4 |
| | | 72 h | 4.6 | 1490 | 6854 | 9.34 | 87.7 |
| | | 96 h | 5.534 | 548 | 3033 | 4.13 | 91.9 |
| | | 120 h | 3.029 | 396 | 1199 | 1.63 | 93.5 |
| | | 144 h | 2.981 | 254 | 757 | 1.03 | 94.5 |
| | | 168 h | 3.908 | 169 | 660 | 0.90 | 95.4 |
| | | | | | | 95.4 | |

| Timepoint (hours) | Cumulative Mean | SD |
|----------------------|--------------------|------|
| 0 h | NA | NA |
| 6 h | 17.2 | 4.4 |
| 12 h | 39.3 | 5.58 |
| 24 h | 61.4 | 5.99 |
| 48 h | 77.9 | 5.58 |
| 72 h | 84.3 | 6.64 |
| 96 h | 87.7 | 6.67 |
| 120 h | 89.5 | 6.55 |
| 144 h | 90.7 | 6.22 |
| 168 h | 91.5 | 6.04 |

Appendix D

Feces Data

Feces Data - Males

| Animal Number | Total H-28548 (ng) | Timepoint (hours) | Sample weight (g) | Concentration H-28548 (ng/g) | Total Amount (ng H-28548) | Percent | Cumulative (%) |
|---------------|--------------------|-------------------|-------------------|------------------------------|---------------------------|---------|----------------|
| 001M | 80620 | 0h | 1.683 | ND | NA | NA | NA |
| | | 6 h | 0.307 | 512 | 157 | 0.19 | 0.19 |
| | | 12 h | 0.396 | 775 | 307 | 0.38 | 0.58 |
| | | 24 h | 2.099 | 95.1 | 200 | 0.25 | 0.82 |
| | | 48 h | 2.381 | 54.7 | 130 | 0.16 | 0.98 |
| | | 72 h | 2.805 | 28.9 | 81 | 0.10 | 1.09 |
| | | 96 h | 2.463 | 13.5 | 33 | 0.04 | 1.13 |
| | | 120 h | 2.869 | <11.1 | NA | NA | 1.13 |
| | | 144 h | 2.447 | <13.0 | NA | NA | 1.13 |
| | | 168 h | 2.563 | <12.5 | NA | NA | 1.13 |
| | | | | | | 1.13 | |

| Animal Number | Total H-28548 (ng) | Timepoint (hours) | Sample weight (g) | Concentration H-28548 (ng/g) | Total Amount (ng H-28548) | Percent | Cumulative (%) |
|---------------|--------------------|-------------------|-------------------|------------------------------|---------------------------|---------|----------------|
| 002M | 78880 | 0h | 1.478 | ND | NA | NA | NA |
| | | 6 h | 0.367 | 122 | 45 | 0.06 | 0.06 |
| | | 12 h | 0.701 | 149 | 104 | 0.13 | 0.19 |
| | | 24 h | 1.694 | 88.9 | 151 | 0.19 | 0.38 |
| | | 48 h | 3.18 | 48.4 | 154 | 0.20 | 0.58 |
| | | 72 h | 3.207 | 29.5 | 95 | 0.12 | 0.70 |
| | | 96 h | 3.302 | 29.4 | 97 | 0.12 | 0.82 |
| | | 120 h | 3.173 | 24.6 | 78 | 0.10 | 0.92 |
| | | 144 h | 2.993 | 12.3 | 37 | 0.05 | 0.96 |
| | | 168 h | 3.291 | 19.7 | 65 | 0.08 | 1.05 |
| | | | | | | 1.05 | |

| Animal Number | Total H-28548 (ng) | Timepoint (hours) | Sample weight (g) | Concentration H-28548 (ng/g) | Total Amount (ng H-28548) | Percent | Cumulative (%) |
|---------------|--------------------|-------------------|-------------------|------------------------------|---------------------------|---------|----------------|
| 003M | 77430 | 0h | 1.598 | ND | NA | NA | NA |
| | | 6 h | 0.626 | 1320 | 826 | 1.07 | 1.07 |
| | | 12 h | 0.553 | 133 | 74 | 0.09 | 1.16 |
| | | 24 h | 1.533 | 56.4 | 86 | 0.11 | 1.27 |
| | | 48 h | 3.188 | 50.8 | 162 | 0.21 | 1.48 |
| | | 72 h | 3.226 | 110 | 355 | 0.46 | 1.94 |
| | | 96 h | 3.354 | 69.2 | 232 | 0.30 | 2.24 |
| | | 120 h | 3.243 | 33.3 | 108 | 0.14 | 2.38 |
| | | 144 h | 3.262 | <9.88 | NA | NA | 2.38 |
| | | 168 h | 3.563 | <8.95 | NA | NA | 2.38 |
| | | | | | | 2.38 | |

Feces Data - Males

| Animal Number | Total H-28548 (ng) | Timepoint (hours) | Sample weight (g) | Concentration H-28548 (ng/g) | Total Amount (ng H-28548) | Percent | Cumulative (%) |
|---------------|--------------------|-------------------|-------------------|------------------------------|---------------------------|---------|----------------|
| 004M | 77140 | 0h | 1.716 | ND | NA | NA | NA |
| | | 6 h | 0.432 | 265 | 114 | 0.15 | 0.15 |
| | | 12 h | 0.945 | 122 | 115 | 0.15 | 0.30 |
| | | 24 h | 2.18 | 120 | 262 | 0.34 | 0.64 |
| | | 48 h | 3.803 | 275 | 1046 | 1.36 | 1.99 |
| | | 72 h | 3.745 | 43.5 | 163 | 0.21 | 2.20 |
| | | 96 h | 3.744 | 73.9 | 277 | 0.36 | 2.56 |
| | | 120 h | 3.734 | 30.7 | 115 | 0.15 | 2.71 |
| | | 144 h | 3.682 | 83.5 | 307 | 0.40 | 3.11 |
| | | 168 h | 4.069 | 76.6 | 312 | 0.40 | 3.51 |
| | | | | | | 3.51 | |

| Animal Number | Total H-28548 (ng) | Timepoint (hours) | Sample weight (g) | Concentration H-28548 (ng/g) | Total Amount (ng H-28548) | Percent | Cumulative (%) |
|---------------|--------------------|-------------------|-------------------|------------------------------|---------------------------|---------|----------------|
| 005M | 78590 | 0h | 1.971 | ND | NA | NA | NA |
| | | 6 h | 0.309 | 194 | 60 | 0.076 | 0.08 |
| | | 12 h | 0.713 | 554 | 395 | 0.50 | 0.58 |
| | | 24 h | 1.718 | 274 | 471 | 0.60 | 1.18 |
| | | 48 h | 3.088 | 126 | 389 | 0.50 | 1.67 |
| | | 72 h | 2.916 | 20.7 | 60 | 0.08 | 1.75 |
| | | 96 h | 2.943 | 19.6 | 58 | 0.073 | 1.82 |
| | | 120 h | 3.199 | 11.5 | 37 | 0.047 | 1.87 |
| | | 144 h | 3.159 | <10.2 | NA | NA | 1.87 |
| | | 168 h | 3.358 | 11.1 | 37 | 0.047 | 1.92 |
| | | | | | | 1.92 | |

| Timepoint (hours) | Cumulative Mean | SD |
|-------------------|-----------------|------|
| 0 h | NA | NA |
| 6 h | 0.31 | 0.43 |
| 12 h | 0.56 | 0.38 |
| 24 h | 0.86 | 0.37 |
| 48 h | 1.34 | 0.56 |
| 72 h | 1.54 | 0.63 |
| 96 h | 1.71 | 0.73 |
| 120 h | 1.80 | 0.78 |
| 144 h | 1.89 | 0.89 |
| 168 h | 2.00 | 1.01 |

Feces Data - Females

| Animal Number | Total H-28548 (ng) | Timepoint (hours) | Sample weight (g) | Concentration H-28548 (ng/g) | Total Amount (ng H-28548) | Percent | Cumulative (%) |
|---------------|--------------------|-------------------|-------------------|------------------------------|---------------------------|---------|----------------|
| 001F | 73808 | 0h | 1.685 | ND | NA | NA | NA |
| | | 6 h | 0.309 | 663 | 205 | 0.28 | 0.28 |
| | | 12 h | 0.544 | 97.4 | 53 | 0.07 | 0.35 |
| | | 24 h | 1.624 | 75.6 | 123 | 0.17 | 0.52 |
| | | 48 h | 3.620 | 99.2 | 359 | 0.49 | 1.01 |
| | | 72 h | 3.480 | 19.7 | 69 | 0.09 | 1.11 |
| | | 96 h | 3.748 | 27.9 | 105 | 0.14 | 1.25 |
| | | 120 h | 3.428 | <10.4 | NA | NA | 1.25 |
| | | 144 h | 3.082 | <10.5 | NA | NA | 1.25 |
| | | 168 h | 3.765 | <8.53 | NA | NA | 1.25 |
| | | | | | | 1.25 | |

| Animal Number | Total H-28548 (ng) | Timepoint (hours) | Sample weight (g) | Concentration H-28548 (ng/g) | Total Amount (ng H-28548) | Percent | Cumulative (%) |
|---------------|--------------------|-------------------|-------------------|------------------------------|---------------------------|---------|----------------|
| 002F | 69600 | 0h | 1.714 | ND | NA | NA | NA |
| | | 6 h | 0.494 | 1640 | 810 | 1.16 | 1.16 |
| | | 12 h | 0.421 | 107 | 45 | 0.06 | 1.23 |
| | | 24 h | 1.454 | 152 | 221 | 0.32 | 1.55 |
| | | 48 h | 3.517 | 37 | 130 | 0.19 | 1.73 |
| | | 72 h | 3.279 | 90.5 | 297 | 0.43 | 2.16 |
| | | 96 h | 3.457 | 17.9 | 62 | 0.09 | 2.25 |
| | | 120 h | 3.576 | 58.8 | 210 | 0.30 | 2.55 |
| | | 144 h | 3.767 | 48.1 | 181 | 0.26 | 2.81 |
| | | 168 h | 3.093 | 13.6 | 42 | 0.06 | 2.87 |
| | | | | | | 2.87 | |

| Animal Number | Total H-28548 (ng) | Timepoint (hours) | Sample weight (g) | Concentration H-28548 (ng/g) | Total Amount (ng H-28548) | Percent | Cumulative (%) |
|---------------|--------------------|-------------------|-------------------|------------------------------|---------------------------|---------|----------------|
| 003F | 69890 | 0h | 1.635 | ND | NA | NA | NA |
| | | 6 h | 0.303 | 182 | 55 | 0.08 | 0.08 |
| | | 12 h | 0.5 | 101 | 51 | 0.07 | 0.15 |
| | | 24 h | 1.178 | 45.9 | 54 | 0.08 | 0.23 |
| | | 48 h | 2.807 | 191 | 536 | 0.77 | 1.00 |
| | | 72 h | 3.128 | 22.2 | 69 | 0.10 | 1.09 |
| | | 96 h | 3.01 | <10.5 | NA | NA | 1.09 |
| | | 120 h | 3.452 | 10.9 | 38 | 0.05 | 1.15 |
| | | 144 h | 2.569 | <12.3 | NA | NA | 1.15 |
| | | 168 h | 3.105 | <10.1 | NA | NA | 1.15 |
| | | | | | | 1.15 | |

Feces Data - Females

| Animal Number | Total H-28548 (ng) | Timepoint (hours) | Sample weight (g) | Concentration H-28548 (ng/g) | Total Amount (ng H-28548) | Percent | Cumulative (%) |
|---------------|--------------------|-------------------|-------------------|------------------------------|---------------------------|---------|----------------|
| 004F | 70760 | 0h | 1.604 | ND | NA | NA | NA |
| | | 6 h | 0.5 | 592 | 296 | 0.42 | 0.42 |
| | | 12 h | 0.895 | 524 | 469 | 0.66 | 1.08 |
| | | 24 h | 1.283 | 134 | 172 | 0.24 | 1.32 |
| | | 48 h | 3.21 | 158 | 507 | 0.72 | 2.04 |
| | | 72 h | 3.42 | 57.5 | 197 | 0.28 | 2.32 |
| | | 96 h | 3.845 | 18.3 | 70 | 0.10 | 2.42 |
| | | 120 h | 3.698 | 29.7 | 110 | 0.16 | 2.57 |
| | | 144 h | 3.603 | 32.8 | 118 | 0.17 | 2.74 |
| | | 168 h | 3.898 | 11.7 | 46 | 0.06 | 2.80 |
| | | | | | | 2.80 | |

| Animal Number | Total H-28548 (ng) | Timepoint (hours) | Sample weight (g) | Concentration H-28548 (ng/g) | Total Amount (ng H-28548) | Percent | Cumulative (%) |
|---------------|--------------------|-------------------|-------------------|------------------------------|---------------------------|---------|----------------|
| 005F | 73370 | 0h | 2.015 | ND | NA | NA | NA |
| | | 6 h | 0.448 | 536 | 240 | 0.33 | 0.33 |
| | | 12 h | 1.051 | 256 | 269 | 0.37 | 0.69 |
| | | 24 h | 1.855 | 63.2 | 117 | 0.16 | 0.85 |
| | | 48 h | 3.817 | 67.5 | 258 | 0.35 | 1.20 |
| | | 72 h | 3.367 | 60.7 | 204 | 0.28 | 1.48 |
| | | 96 h | 3.699 | <8.35 | NA | NA | 1.48 |
| | | 120 h | 3.217 | <9.75 | NA | NA | 1.48 |
| | | 144 h | 3.495 | <9.20 | NA | NA | 1.48 |
| | | 168 h | 3.346 | <9.63 | NA | NA | 1.48 |
| | | | | | | 1.48 | |

| Timepoint (hours) | Cumulative Mean | SD |
|-------------------|-----------------|------|
| 0 h | NA | NA |
| 6 h | NA | NA |
| 12 h | 0.70 | 0.46 |
| 24 h | 0.89 | 0.55 |
| 48 h | 1.40 | 0.47 |
| 72 h | 1.63 | 0.58 |
| 96 h | 1.70 | 0.60 |
| 120 h | 1.80 | 0.71 |
| 144 h | 1.89 | 0.82 |
| 168 h | 1.91 | 0.85 |

Appendix E
Cage Wash Data

Cage Wash Data - 168 hours

| Animal Number | Total H-28548 (ng) | Timepoint (hours) | Sample Weight (g) | Concentration H-28548 (ng/g) | Total Amount (ng H-28548) | Percent |
|------------------|--------------------------|----------------------|-------------------------|------------------------------------|------------------------------|---------|
| 001M | 80620 | 168 h | 274.506 | 32.3 | 8867 | 11.0 |
| 002M | 78880 | 168 h | 274.514 | 30.8 | 8455 | 10.7 |
| 003M | 77430 | 168 h | 214.319 | 24.4 | 5229 | 6.75 |
| 004M | 77140 | 168 h | 207.445 | 55.7 | 11555 | 15.0 |
| 005M | 78590 | 168 h | 214.205 | 17.4 | 3727 | 4.74 |
| | | | | | Mean | 9.64 |
| | | | | | SD | 3.99 |

| Animal Number | Total H-28548 (ng) | Timepoint (hours) | Sample Weight (g) | Concentration H-28548 (ng/g) | Total Amount (ng H-28548) | Percent |
|------------------|--------------------------|----------------------|-------------------------|------------------------------------|------------------------------|---------|
| 001F | 73080 | 168 h | 227.935 | 12.9 | 2940 | 4.02 |
| 002F | 69600 | 168 h | 230.137 | 14.4 | 3314 | 4.76 |
| 003F | 69890 | 168 h | 257.292 | 15.3 | 3937 | 5.63 |
| 004F | 70760 | 168 h | 212.796 | 39.3 | 8363 | 11.8 |
| 005F | 73370 | 168 h | 244.357 | 15.1 | 3690 | 5.03 |
| | | | | | Mean | 6.25 |
| | | | | | SD | 3.16 |

Appendix F

Material Balance

Material Balance

| | | 001M | 002M | 003M | 004M | 005M | Mean | SD |
|-----------|----------|-------|-------|-------|-------|-------|-------|------|
| urine | 6 h | 20.9 | 18.5 | 11.7 | 9.21 | 10.4 | 14.1 | 5.19 |
| urine | 12 h | 9.32 | 13.0 | 20.3 | 13.5 | 27.2 | 16.7 | 7.12 |
| urine | 24 h | 29.5 | 21.3 | 22.3 | 23.1 | 24.0 | 24.1 | 3.20 |
| urine | 48 h | 16.7 | 17.7 | 24.6 | 13.7 | 16.5 | 17.8 | 4.06 |
| urine | 72 h | 6.19 | 8.58 | 6.49 | 8.35 | 6.60 | 7.24 | 1.13 |
| urine | 96 h | 4.07 | 4.45 | 4.37 | 4.21 | 3.52 | 4.12 | 0.37 |
| urine | 120 h | 2.38 | 2.74 | 2.46 | 2.26 | 2.26 | 2.42 | 0.20 |
| urine | 144 h | 1.41 | 1.66 | 1.76 | 1.83 | 1.98 | 1.73 | 0.21 |
| urine | 168 h | 1.20 | 1.30 | 1.27 | 1.71 | 1.06 | 1.31 | 0.25 |
| | Subtotal | 91.7 | 89.2 | 95.3 | 77.9 | 93.6 | 89.5 | 6.91 |
| feces | 6 h | 0.19 | 0.06 | 1.07 | 0.15 | 0.08 | 0.31 | 0.43 |
| feces | 12 h | 0.38 | 0.13 | 0.09 | 0.15 | 0.50 | 0.25 | 0.18 |
| feces | 24 h | 0.25 | 0.19 | 0.11 | 0.34 | 0.60 | 0.30 | 0.19 |
| feces | 48 h | 0.16 | 0.20 | 0.21 | 1.36 | 0.50 | 0.48 | 0.51 |
| feces | 72 h | 0.10 | 0.12 | 0.458 | 0.21 | 0.08 | 0.19 | 0.16 |
| feces | 96 h | 0.04 | 0.12 | 0.30 | 0.36 | 0.073 | 0.18 | 0.14 |
| feces | 120 h | <LOQ | 0.10 | 0.14 | 0.15 | 0.047 | 0.11 | 0.05 |
| feces | 144 h | <LOQ | 0.05 | <LOQ | 0.399 | <LOQ | 0.22 | 0.25 |
| feces | 168 h | <LOQ | 0.08 | <LOQ | 0.40 | 0.05 | 0.18 | 0.20 |
| | Subtotal | 1.13 | 1.05 | 2.38 | 3.51 | 1.92 | 2.00 | 1.01 |
| cage wash | 168 h | 11.00 | 10.72 | 6.75 | 14.98 | 4.74 | 9.64 | 3.99 |
| | Total | 103.8 | 101.0 | 104.4 | 96.3 | 100.3 | 101.2 | 3.22 |
| | | 001F | 002F | 003F | 004F | 005F | Mean | SD |
| urine | 6 h | 12.4 | 19.3 | 22.7 | 18.5 | 13.0 | 17.2 | 4.41 |
| urine | 12 h | 24.0 | 19.1 | 26.4 | 17.7 | 23.4 | 22.1 | 3.60 |
| urine | 24 h | 26.8 | 21.5 | 21.6 | 18.9 | 21.7 | 22.1 | 2.88 |
| urine | 48 h | 18.11 | 17.5 | 12.8 | 13.8 | 20.3 | 16.5 | 3.11 |
| urine | 72 h | 5.15 | 5.16 | 7.53 | 4.87 | 9.34 | 6.41 | 1.96 |
| urine | 96 h | 4.03 | 3.05 | 2.49 | 3.24 | 4.13 | 3.39 | 0.69 |
| urine | 120 h | 1.55 | 1.72 | 1.82 | 2.00 | 1.63 | 1.74 | 0.17 |
| urine | 144 h | 0.79 | 2.05 | 0.74 | 1.46 | 1.03 | 1.22 | 0.55 |
| urine | 168 h | 0.61 | 1.25 | 0.49 | 1.04 | 0.90 | 0.86 | 0.31 |
| | Subtotal | 93.4 | 90.7 | 96.7 | 81.5 | 95.4 | 91.5 | 6.04 |
| feces | 6 h | 0.28 | 1.16 | 0.08 | 0.42 | 0.33 | 0.45 | NA |
| feces | 12 h | 0.07 | 0.06 | 0.07 | 0.66 | 0.37 | 0.25 | 0.27 |
| feces | 24 h | 0.17 | 0.32 | 0.08 | 0.24 | 0.16 | 0.19 | 0.09 |
| feces | 48 h | 0.49 | 0.19 | 0.77 | 0.72 | 0.35 | 0.50 | 0.24 |
| feces | 72 h | 0.09 | 0.43 | 0.10 | 0.28 | 0.28 | 0.24 | 0.14 |
| feces | 96 h | 0.14 | 0.09 | <LOQ | 0.10 | <LOQ | 0.11 | 0.03 |
| feces | 120 h | <LOQ | 0.302 | 0.05 | 0.16 | <LOQ | 0.17 | 0.12 |
| feces | 144 h | <LOQ | 0.260 | <LOQ | 0.17 | <LOQ | 0.21 | 0.07 |
| feces | 168 h | <LOQ | 0.060 | <LOQ | 0.064 | <LOQ | 0.06 | 0.00 |
| | Subtotal | 1.25 | 2.87 | 1.15 | 2.80 | 1.48 | 1.91 | 0.85 |
| cage wash | 168 h | 4.02 | 4.76 | 5.63 | 11.8 | 5.03 | 6.25 | 3.16 |
| | Total | 98.7 | 98.3 | 103.4 | 96.2 | 102.0 | 99.7 | 2.95 |

Appendix G

Elimination Half-Life

Elimination Half-Life

OriginLab v7.0220, interpolation of mean urinary excretion data; interpolated data points every 3 hours from 0 to 168 hours (56 data points)

T_{1/2} Males: 21 hours
T_{1/2} Females: 18 hours

Bolded/underlined values (*) identify the elimination half-lives (≥50% of the administered dose) and associated cumulative percent of H-28548 in urine

| Time, post-dose (hours) | Cumulative percent of H-28548 eliminated in urine | |
|----------------------------|---|-------------------------|
| | Male | Female |
| 0 | -2.6 | -4.9 |
| 3.05455 | 5.90182 | 6.35091 |
| 6.10909 | 14.40364 | 17.60182 |
| 9.16364 | 22.90545 | 28.85273 |
| 12.21818 | 31.23818 | 39.70182 |
| 15.27273 | 37.37273 | 45.32727 |
| <u>18.32727*</u> | 43.50727 | <u>50.95273*</u> |
| <u>21.38182*</u> | <u>49.64182*</u> | 56.57818 |
| 24.43636 | 55.22364 | 61.7 |
| 27.49091 | 57.48909 | 63.8 |
| 30.54545 | 59.75455 | 65.9 |
| 33.6 | 62.02 | 68 |
| 36.65455 | 64.28545 | 70.1 |
| 39.70909 | 66.55091 | 72.2 |
| 42.76364 | 68.81636 | 74.3 |
| 45.81818 | 71.08182 | 76.4 |
| 48.87273 | 72.96545 | 78.13273 |
| 51.92727 | 73.89455 | 78.94727 |
| 54.98182 | 74.82364 | 79.76182 |
| 58.03636 | 75.75273 | 80.57636 |
| 61.09091 | 76.68182 | 81.39091 |
| 64.14545 | 77.61091 | 82.20545 |
| 67.2 | 78.54 | 83.02 |
| 70.25455 | 79.46909 | 83.83455 |
| 73.30909 | 80.22364 | 84.48545 |
| 76.36364 | 80.74545 | 84.91818 |
| 79.41818 | 81.26727 | 85.35091 |
| 82.47273 | 81.78909 | 85.78364 |
| 85.52727 | 82.31091 | 86.21636 |
| 88.58182 | 82.83273 | 86.64909 |
| 91.63636 | 83.35455 | 87.08182 |
| 94.69091 | 83.87636 | 87.51455 |
| 97.74545 | 84.27455 | 87.83091 |
| 100.8 | 84.58 | 88.06 |
| 103.85455 | 84.88545 | 88.28909 |
| 106.90909 | 85.19091 | 88.51818 |
| 109.96364 | 85.49636 | 88.74727 |
| 113.01818 | 85.80182 | 88.97636 |
| 116.07273 | 86.10727 | 89.20545 |
| 119.12727 | 86.41273 | 89.43455 |
| 122.18182 | 86.65455 | 89.60909 |
| 125.23636 | 86.87091 | 89.76182 |
| 128.29091 | 87.08727 | 89.91455 |
| 131.34545 | 87.30364 | 90.06727 |
| 134.4 | 87.52 | 90.22 |
| 137.45455 | 87.73636 | 90.37273 |
| 140.50909 | 87.95273 | 90.52545 |
| 143.56364 | 88.16909 | 90.67818 |
| 146.61818 | 88.34182 | 90.78727 |
| 149.67273 | 88.50727 | 90.88909 |
| 152.72727 | 88.67273 | 90.99091 |
| 155.78182 | 88.83818 | 91.09273 |
| 158.83636 | 89.00364 | 91.19455 |

| Time, post-dose (hours) | Cumulative percent of H-28548 eliminated in urine | |
|----------------------------|---|----------|
| | Male | Female |
| 161.89091 | 89.16909 | 91.29636 |
| 164.94545 | 89.33455 | 91.39818 |
| 168 | 89.5 | 91.5 |